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AUTHORS ALONE ARE RESPONSIBLE FOR OPINIONS EXPRESSED IN THEIR CONTRIBUTIONS

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BULLETIN OF
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JULY 1938

VIRUSES AND VIRUS DISEASES*

Twentieth Century Version of the De Novo *Origin of Infectious Agents and Its Significance in Relation to the Control of Disease*

THOMAS M. RIVERS

HERMANN M. BIGGS† while a candidate for the degree of Bachelor of Arts at Cornell secured a leave of absence for a few months in order to study at Bellevue Hospital Medical College. The influence of that experience is reflected in his baccalaureate thesis, "Sanitary Regulations and the Duty of the State in Regard to Public Hygiene." For the most part, statements in his thesis hold today as well as they did in 1882. However, the accuracy of one remark would be questioned at the present time; in fact, Biggs himself later did much work to show that such a statement was inaccurate. The one referred to is: "It is now universally acknowledged by all medical authorities that a number of these diseases such as cholera, typhoid and typhus fever and diphtheria may be generated *de novo* in certain localities from the use of impure water and the existence of unsanitary conditions, and thence are scattered by contagion; and it is now believed by some of the best medical writers that many if not all of the remainder of these diseases such as smallpox,

* From the Hospital of The Rockefeller Institute for Medical Research.

† Winslow, C.-E.A. *The life of Hermann M. Biggs.* Philadelphia, Lea & Febiger, 1929.

scarlet fever and measles were originally among the so-called filth diseases, and originated from essentially the same conditions as the others. If this is true we are forced to the conclusion that all of these zymotic diseases the most terrible known to man, that have wrought so much misery, desolation and death in the world are the direct result of man's own carelessness and negligence, the inevitable penalty of the violation of sanitary laws."

Again in 1885, after Biggs had obtained his medical degree, he and Breneman made a similar statement in connection with the investigation of an epidemic of typhoid in Plymouth, Pennsylvania. In their report the following remark appears: "If it be possible for typhoid fever to originate *de novo* in filthy surroundings and in the use of polluted water, no more remote point need be looked for as the starting point of the disease which spread from a single patient in this house to hundreds in the village . . . Everything points therefore to the conclusion that the disease which spread from this house also originated there."

It is not surprising that Biggs had such thoughts at that time even though Pasteur had finished his work on spontaneous generation and the germ theory of infectious diseases was gradually being accepted. Ideas set forth by Austin Flint in 1881 in his fifth edition of *Principles and Practice of Medicine*, which was prepared with the assistance of Welch in the field of pathology and which was used by Biggs as a text-book, undoubtedly influenced him considerably. For instance, in regard to diphtheria, Flint made the following statement: "Specific cases occur under circumstances which seem to render it impossible that a contagium was involved. The number of such instances justifies the opinion that, if the disease be contagious, it may be also produced without the agency of contagium." Furthermore, in regard to typhoid, Flint said: "Whether the production of the causative germs is ever independent of the disease is not settled; in other words, it is an unsettled question whether the disease is ever produced irrespective of communicability." Carriers of infectious agents and missed cases had not yet been recognized; consequently it was difficult at that time to explain adequately the spread of many infectious diseases.

Biggs died in 1923 before the present activity in the study of virus diseases was far advanced. If he had lived longer, it is obvious, in view of his early statements regarding the *de novo* origin of certain maladies, that he would have been greatly interested in the present day discussions

of the origin and nature of viruses. Before commenting on activities in the virus field that deal with this subject, I shall recite some historical facts about the controversy over spontaneous generation and xenogenesis or heterogenesis in order to provide a proper background.

Since Thomas Huxley's presidential address on "Biogenesis and Abiogenesis" to the British Association for the Advancement of Science in 1870, a number of similar discussions have appeared. In 1928, before the Pathological Section of the Royal Society of Medicine, Boycott gave his presidential address, "The Transition from Live to Dead: The Nature of Filterable Viruses"; in 1930, before the Pacific Northwest Medical Association, I discussed "Spontaneous Generation and Filterable Viruses"; in 1935, Sir Henry Dale's Huxley Memorial Lecture was on "Viruses and Heterogenesis: An Old Problem in a New Form." The question is so important and of such universal interest that I shall again undertake its discussion in spite of the risk of undue repetition.

The description of Francesco Redi's work on the generation of insects appeared in 1668*; the current opinion at that time concerning the origin of such living things was expressed by Redi in the following manner:

"After a long period of fertility, during which time many monstrous and marvelous generations were brought forth, the Earth Mother became at last exhausted and sterile and lost her power of producing man and the larger animals, still she retained enough vigor to bring forth (besides plants, that are presumed to be generated spontaneously) certain small creatures, such as flies, wasps, spiders, ants, scorpions, and all other terrestrial and aerial insects. The schools both ancient and modern all agree in this, and constantly teach that the Earth has continued to produce these creatures and will produce them as long as she exists. They do not, however, agree as to the manner in which these insects are generated, nor how life is communicated to them; for they say that not only does the Earth possess this occult power, but that it is possessed by animals, living and dead, also by all things produced from the Earth, and finally by those which are about to decay and return to dust. Hence others have claimed putrefaction itself to be the all potent cause of generation . . . "

Alexander Ross, an English teacher and preacher, in his book, "Arcana Microcosmi; . . . with a refutation of Dr. Browne's Vulgar

* Bigelow's English translation (1909) and Cole's essay (Cole, R. Francesco Redi (1626-1697) *Ann. M. Hist.*, 1926, *8*:347) on Redi are the sources of information and quotations.

Errors", in 1651 voiced an opinion of Sir Thomas Browne's doubt as to "whether mice may be bred by putrefaction"; and this opinion, which follows immediately, further illuminates the ideas held by Redi's contemporaries regarding spontaneous generation:

"So may he doubt whether in cheese and timber worms are generated; or if beetles and wasps in cow dung; or if butterflies, locusts, grasshoppers, shell fish, snails, eels, and such like, be procreated of putrid matter, which is apt to receive the form of that creature to which it is by formative power disposed. To question this is to question reason, sense, and experience. If he doubts this let him go to Egypt, and there he will find the fields swarming with mice begot of the mud of Nylus, to the great calamity of the inhabitants."

From the preceding remarks it is obvious that most of Redi's contemporaries believed that living things arose spontaneously not only from dead material but also from putrid matter. That death is the source of life also had Biblical sanction. Saint Paul in one of his eloquent outbursts exclaimed: "Thou fool, that which thou sowest is not quickened, except it die" (Corinthians XV: 36). Indeed, Biblical sanction of the idea that bees originate in carcasses of dead bulls and lions was found in the story of Sampson, who, as you remember, killed a lion, and, later when he passed that way again, saw in its carcass a swarm of bees with honey. From this experience it has been affirmed that Sampson conceived the riddle that puzzled the Philistines: "Out of the eater came forth meat, and out of the strong came forth sweetness" (Judges XIV: 14).

In spite of the general belief in spontaneous generation, "sanctioned alike by antiquity and by authority", Redi proceeded to show by experiments that worms are not spontaneously engendered in the flesh of dead animals. He accomplished this by dividing bits of fresh meat into two portions, one of which was left open to the air and insects, while the other portion was placed in a vessel with a large mouth, over which was stretched gauze that permitted the entry of air and prevented the access of insects. The meat protected by the gauze remained free of maggots, while in the unprotected portion numerous worms appeared. Many experiments of this nature were performed, and from data thus obtained, Redi concluded:

"Although content to be corrected by anyone wiser than myself, if I should make erroneous statements, I shall express my belief that the Earth, after having brought forth the first plants and animals at the

beginning by order of the Supreme and Omnipotent Creator, has never since produced any kind of plants or animals, either perfect or imperfect; and everything which we know in past or present time she has produced, came solely from true seeds of the plants and animals themselves, which thus, through means of their own, preserve their species."

Redi was a firm believer in biogenesis; however, he held that there were two kinds, homogenesis in which the offspring is like the parent, and xenogenesis or heterogenesis in which the offspring is completely and permanently unlike the parent. By means of the latter mode, he explained the origin of grubs in plant galls, and nearly a century passed before this explanation was definitely shown to be incorrect. Indeed, it was not until during the first half of the nineteenth century that the idea that intestinal worms are generated by their hosts through the process of xenogenesis or heterogenesis was finally and completely discredited. During the latter part of the seventeenth century and the early years of the eighteenth, the theory of spontaneous generation seems to have fallen into disrepute. The invention of the microscope, however, at the end of the seventeenth century which enabled investigators to see bacteria, brought fresh arguments in favor of spontaneous generation, and about the middle of the eighteenth century the problem again became of interest to scientists.*

Needham and Buffon worked in support of the conception of spontaneous generation; Spallanzani opposed it. At this time it is not worthwhile to describe in detail their experiments; nevertheless, it does seem advisable to state briefly the viewpoint of the workers first mentioned. According to them, "there are certain primitive and incorruptible parts common to animals and vegetables." These indestructible parts or "molecules" are liberated and, after thoroughly working the putrid material by means of their activity, appropriate to themselves new particles, finally to form "by their reunion, a multitude of little organized bodies, of which some, like earthworms and fungi, seem to be fair-sized animals or vegetables, but of which others, in almost infinite numbers, can only be seen through the microscope."

According to Huxley, the above doctrine is not identical with that of abiogenesis or spontaneous generation and should be classified under xenogenesis or heterogenesis. He clarified the doctrine in the following manner: "On this hypothesis, a piece of beef or a handful of hay, is

* Vallery-Radot, R. *Life of Pasteur*, translated by Mrs. R. L. Devonshire. London, Constable, 1911.

dead only in a limited sense. The beef is dead ox, and the hay is dead grass; but the 'organic molecules' of the beef or the hay are not dead, but are ready to manifest their vitality as soon as the bovine or herbaceous shrouds in which they are imprisoned are rent by the macerating action of water."

Following the controversy of Spallanzani with Needham and Buffon, the theory of spontaneous generation had its good and bad moments for approximately a hundred years, that is to say until 1858, at which time it was brought to the front again by Pouchet in a "Note on Vegetable and Animal Proto-organisms Spontaneously Generated in Artificial Air and Oxygen Gas." At this point Pasteur appeared on the scene, and, in 1860 he wrote: "I am pursuing as best I can these studies on fermentation which are of greatest interest, connected as they are with the impenetrable mystery of Life and Death. I am hoping to mark a decisive step very soon by solving, without the least confusion, the celebrated question of spontaneous generation. Already I could speak, but I want to push my experiment further. There is so much obscurity, together with so much passion, on both sides, that I shall require the accuracy of an arithmetical problem to convince my opponents by my conclusions. I intend to attain even that."

Pasteur's experiments seemed at the time to overthrow completely the theory of spontaneous generation. After this work he turned his attention to other problems. Thus he wrote to a friend: "What would be most desirable would be to push those studies (that is, on spontaneous generation) far enough to prepare the road for a serious research into the origin of various diseases." As is well known, he pursued the matter and subsequently made many notable discoveries concerning the relation of bacteria to infectious disease. Indeed, he, Koch and others so firmly established the fact that small living animals and plants, *i.e.*, protozoa, fungi, bacteria, and spirochetes, are capable of producing disease that in due course of time it became a heresy for one to suggest that an infectious disease might be caused in another manner or by agents wholly unlike those just mentioned.

Redi, in 1668, showed that insects do not arise spontaneously, and Pasteur, two centuries later, demonstrated that small living organisms, *e.g.*, bacteria, visible only by means of the microscope, yet capable of producing disease, do not arise *de novo*. Now we come to another interesting and stormy period in the development of knowledge concern-

ing the origin and nature of a certain group of disease-producing agents.

In 1892, Iwanowski observed that the etiological agent of mosaic disease of tobacco plants is capable of passing through earthen-ware filters impervious to ordinary bacteria. This observation was confirmed in 1898 by Beijerinck. Also in 1898, Loeffler and Frosch showed that the agent responsible for foot-and-mouth disease in cattle is capable of traversing bacteria-tight filters and is invisible under the microscope. In the same year Sanarelli demonstrated similar facts regarding the causative agent of an infectious tumor of rabbits. In rapid succession, many disease-producing agents, *e.g.*, those causing fowl-pox, vaccinia, smallpox, hydrophobia, and yellow fever, were found to be filterable and invisible. Thus, we come to the period signalized by a group of disease-inciting agents to which many names have been applied, *e.g.*, viruses, filterable viruses, ultraviruses, ultramicroscopic viruses, ultramicrobes, and inframicrobes.

What is the nature of these active agents? In view of the fact that they multiply or increase in amount and are capable of producing diseases, most workers in the past have been led by analogy to assume that the agents represent the midgets of the microbial world, *i.e.*, exceedingly small protozoa, fungi, bacteria, or spirochetes. From the very beginning, however, certain workers have registered protests against such assumptions. For instance, Beijerinck considered the cause of mosaic to be a living contagious fluid. Woods held it to be an enzyme. Sanfeleci, in 1914, was led to believe from the results of experiments that the cause of fowl-pox is an inanimate poison capable of attacking normal cells and of inciting in them the production of a poison of a similar nature which in turn could attack other normal cells. Thus he described his idea of how a lifeless agent might be passed in series being reproduced indefinitely.

From 1898 to 1915, investigators, with few exceptions, considered the filterable viruses to be small living organisms. Furthermore, a host of workers claimed that they could cultivate them on simple lifeless media. These claims, however, have not been substantiated, and at present most investigators in this field believe that viruses increase or are regenerated only in the presence of susceptible living host cells. Then came the work of Twort and d'Herelle on bacteriophagy, a phenomenon in which bacteria are disrupted or lysed by an agent regenerated during the course of the phenomenon and transmissible in series to susceptible

bacteria. Both of these men consider the phenomenon an acute infectious disease of bacteria. Twort says that the agent causing this malady belongs "somewhere in the vast field of life more lowly organized than the bacterium" and suggests that "it may be living protoplasm that forms no definite individuals, or are enzymes with power of growth", or finally, a material arising spontaneously in normal bacteria. On the other hand, d'Herelle firmly believes that the bacteriophage is a living agent and speaks of it in the following manner: "The protobe, then, penetrates the peripheral zone of the bacterium and multiplies within the bacterial protoplasm, forming a colony composed of a number of elements varying with the conditions of the moment. When process of multiplication is completed, the parasitized bacterium ruptures and undergoes a sudden dissolution. This liberates the young protobes and each of the latter is then ready to repeat the process and parasitizes the nearest bacterium. Thus the cycle begins anew, and continues until all the bacteria present in the medium have disappeared."

Other workers have taken up the problem and numerous ideas concerning bacteriophages now exist. Bordet does not believe that they are living autonomous agents. According to Bail, the phenomenon of bacteriophagy is due to the fact that bacterial chromosomes lose their anabolic function. Thus, cells with chromosomes possessing only the function of katabolism undergo dissolution, liberating the imperfect chromosomes which are then capable of initiating similar vitiations in other bacteria. Northrop appears to be convinced, on the basis of his own experimental findings, that the infectious units of the strain of staphylococcus bacteriophage with which he is working are large nucleoprotein molecules which are regenerated in infected bacteria in a manner "more simply explained by analogy with the autocatalytic formation of pepsin and trypsin than by analogy with the far more complicated system of living organisms."

A number of workers consider that they have been able to demonstrate that bacteriophages arise spontaneously in normal bacteria. Nevertheless, Bronfenbrenner, an ardent advocate of the idea that the agents of transmissible lysis of bacteria are bacterial products, believes that "the reports concerning the successful production of phage from bacteria without the intermediary of other biological materials have thus far not been entirely convincing."

The bacteriophage is a filterable agent much smaller than the bacteria

attacked by it, seems to be incapable of increasing in amount in the absence of susceptible bacteria, exhibits a high degree of specificity which in certain instances can be altered by adaptation, gives rise to a specific flocculation reaction when brought in contact with antisera prepared in rabbits; such antisera also neutralize its activity. There are many strains of bacteriophage; their resistance to physical and chemical forces varies with the strain. The different strains are not serologically identical, but all of them are serologically different from the proteins of their bacterial hosts. They vary in size; large ones are 60 mμ in diameter, a size compatible with the idea that they are very small living organisms; the small ones possess a diameter of 10 mμ, a size similar to that of certain protein molecules. For instance, molecules of edestin and hemocyanin are, respectively, 10 and 24 mμ in diameter. No one has shown that the bacteriophages possess independent metabolic activities. Are they alive? Some contend that they are inanimate agents, while others believe them to be living autonomous organisms.

Although d'Herelle is convinced that the bacteriophages are living autonomous organisms, he has been forced to discard the idea that the cell is the unit of life and speaks of the matter in relation to viruses in the following manner:

"For many years we have spoken of the ultraviruses and we will see that these beings can only be constituted by a simple colloidal micella. But these beings are necessarily alive for they possess all the characteristics of living organisms . . . To state that a substance which possesses all of the characteristics of living beings . . . cannot be a living being is incomprehensible nonsense.

"A being composed of a micella cannot be a cellular being, and as a consequence the cellular concept of life is necessarily false for it fails to take into consideration the facts of the case. It is thus necessary to revise the theory to provide an hypothesis which conforms to the fact, that is, that life—the sum total of the powers of assimilation and of adaptation, is the result, not of a cellular organization, but of a special physiochemical constitution of matter."

Recently Gratia has expressed a similar idea in the following statement: "Viruses and bacteriophages reproduce themselves in a manner similar to autonomous cells, without doubt very simple cells, reduced to a micella, but still similar to micro-organisms." A micella, as you recall is one of the hypothetical aggregations of molecules of which, according

to Naegeli's micellar theory, organized bodies are composed. Indeed, it has been assumed that micellae have a crystalline structure and are capable of increase or diminution without change in chemical structure. This is a very interesting assumption in view of the recent work of Stanley on the crystallization of tobacco mosaic virus.

The possibility that the unit of autonomous life is smaller than the cell must be looked upon as an interesting hypothesis rather than as a fact. Furthermore, others are not so positive as d'Herelle in their statements concerning life. For instance, Henry Fairfield Osborn did not speak for himself alone when at the end of his book, *The Origin and Evolution of Life* he said:

"The difference between the non-living world and the living world seems like a vast chasm when we think of a very high organism like man . . . But the difference between primordial earth, water and atmosphere, and the lowliest known organisms which secure their energy directly from simple chemical compounds is not so vast a chasm that we need despair of bridging it some day by solving at least one problem as to the actual nature of life, namely, whether it is solely physico-chemical in its energies, or whether it includes a *plus* energy or element which may have distinguished life from the beginning."

Certain highly critical individuals may ask, "Why devote so much time to the bacteriophages in a discussion of viruses?" To the initiate they are as truly viruses as are the other active agents placed in the virus group. However, to avoid criticism let us consider for a moment in such relation the recent work on the virus that causes mosaic disease in tobacco plants. Surely no one can offer criticism to this, because this agent was the first filterable virus to be discovered and around which the virus group has been formed.

Tobacco mosaic virus produces a transmissible disease so highly infectious that it may cause epidemics in fields of tobacco plants. Transmission to healthy plants may take place by means of bites of certain insects that have fed on infected plants or by inoculation of infectious material into areas of leaves injured by rubbing or by puncture wounds. In an infected plant the virus rapidly increases in amount, but it has never been cultivated apart from susceptible living host cells. Infectious units of the virus are not visible under the microscope. The virus remains active for long periods of time in cigars, cigarettes, and chewing tobacco. The thermal resistance of the agent is greater than that of most ordinary

bacteria, but no greater than that of certain thermophilic micro-organisms. Specific precipitating and neutralizing antisera for the virus can be prepared by the injection of the active agent into rabbits. On the assumption that an infectious unit of this agent is round or oval, the diameter of a unit has been estimated by means of centrifugation and filtration to be approximately 30 mμ. However, considerable evidence has been brought to show that the infectious units are not round objects but oblong particles with a length tenfold greater than the width. Recently Stanley and other workers have obtained large amounts of a purified nucleoprotein from plants infected with the virus and have been able to crystallize and recrystallize it repeatedly. This nucleoprotein, made up of very large molecules, is not present in normal plants, constitutes the major portion of the protein in diseased plants, and possesses all the activities and properties of the virus. Stanley has on many occasions tried to separate this protein from the virus but has been unable to do so, and, in view of this fact, believes that molecules of the heavy protein represent infectious units of virus. Similar observations have been made by Stanley and other investigators in connection with work on several other plant viruses.

Bacteriophages and the agent that causes tobacco mosaic may be considered viruses of medium and small size. Now a few words should be said regarding some of the large viruses, *e.g.*, those causing smallpox, vaccinia, fowl-pox, infectious myxomatosis of rabbits, infectious fibromas, and psittacosis. The infectious agents of these maladies are represented by round or oval particles, elementary bodies, possessing diameters of 150 to 250 mμ. The bodies can be made visible in ordinary light by means of proper stains. They do not multiply in the absence of living host cells but do so when brought into contact with them under proper conditions. No independent metabolic activity in them has been demonstrated. They are specifically agglutinated and their activity is specifically inhibited by homologous antisera. For some of them multiple antigens have been shown to exist. Washed elementary bodies on analysis yield protein, fat, carbohydrates, and ash. It appears that their density is considerably less than that of ordinary proteins. Berry and his coworkers have transformed fibroma virus into myxoma virus by the injection into animals of a mixture of active fibroma virus and inactivated myxoma elementary bodies, a technique similar to that used by Griffith to transform one type of pneumococcus into another. In view of the

work done on elementary bodies, many workers, particularly the British, are inclined to look upon them as minute living organisms. In any event, it is safe to say that these bodies do not conform to the ideas usually held regarding ordinary protein molecules, and, until further evidence to the contrary is brought, one should consider that they are more complex in nature than are the molecules of ordinary proteins and the heavy proteins so far described.

In view of the wide interest taken in the recent work on virus heavy proteins, it is justifiable to quote several comments made by Stanley in order to show that he has not been able to define completely the nature of the viruses with which he is working:

"Although, with the sole exception of size, these large viruses appear to be similar to the small viruses, insufficient experimental evidence is available at present to justify the definite conclusion that they either are or are not protein molecules. In view of the results with some of the smaller viruses, there should be no objection to using, as a working hypothesis, the idea that the largest viruses are also macromolecules."

"The virus proteins not only bridge the gap between molecules and artificially prepared colloidal particles, but they also bridge the gap between molecules and living organisms. The smaller virus proteins are the same order of size as the largest hemocyanin molecules, whereas the largest viruses are of the same order of size as admittedly living organisms . . . "

Because of this reproductive power, they have been called autocatalysts. Although this is technically incorrect in that the reaction is very probably not that of a true autocatalysis, the term was used because the net result is somewhat similar to that of an autocatalytic reaction. However, the mechanism by means of which a molecule in a specific living cell is able to cause the production of identical or similar molecules is unknown. It may be similar to the mechanism which the geneticists postulate for the production of genes. The virus protein molecule may be able to cause smaller molecules already present in the cell, or smaller molecules produced by the cell as a result of the presence of the virus molecule, to organize and combine to form a new virus molecule."

"It is obvious that as molecules they possess properties which differ somewhat from the ordinary properties of molecules and that as organisms they possess properties which differ somewhat from the ordinary properties of organisms."

"Since the virus proteins possess virus activity and certain properties characteristic of organisms, as well as the properties of molecules, any attempt at this time to classify them definitely as molecules or as organisms should be one solely of convenience."

It is not clear what name should be applied to the kind of regeneration or reproduction that has been described by Stanley for viruses. Some investigators probably would call it heterogenesis. On the other hand, if the virus proteins are not alive, no obvious reason is apparent for supposing anything happens in the process of their production more mysterious than the biochemical reactions essential for the formation of proteins. Indeed, the formation of protein is sufficiently mysterious and what actually goes on in nature during such a process is by no means fully understood.

The fact that viruses have not been cultivated in the absence of living susceptible cells has impressed a number of workers to such an extent that they have been led to attempt the *de novo* fabrication of viruses through the altered activity of normal cells. Murphy, Carrel, Fischer, and others have taken an active part in this type of work which has been done largely in the virus field related to filterable tumor-producing agents.

The usual procedure has been to subject "normal" tissues to certain physical and chemical agents after which the treated materials are injected into "normal" fowls, or to inject coal tar or some of its derivatives into chickens. Following such procedures a certain number of tumors, transmissible in series by cell-free filtrates, have been obtained. These experiments are interesting and may be significant; but one should be highly critical of them, because none of the workers has shown that a latent virus was not present in the so-called normal tissues or in the so-called normal chickens receiving them after treatment. Such a critical attitude is amply justified by the results of my work on Virus III infections in rabbits, the work of Cole and Kuttner on salivary-gland disease of guinea pigs, and that of Traub on lymphocytic choriomeningitis in mice.

From what has been said regarding the viruses, it is now evident why spontaneous generation was considered earlier in the talk. It was done to show present problems in their historic relation to this idea. Slowly scientists have been descending the scale of life as regards size, and at each step have settled to their satisfaction the question of spon-

taneous generation. Now they are face to face with viruses, the "infinitely small in biology." Although no investigator working in the virus field has claimed to have generated living organisms from inanimate matter, it appears that a few believe that they have by certain manipulations induced cells to yield substances which possess some of the attributes of life, notably that of increasing without limit.

The nature of viruses is still an open question. Nor is it essential that all be alike in nature. If there be a sharp demarcation between life and death, then scientists, investigating the nature of viruses, are working near the line that separates infinitely small living organisms from inanimate active agents. Such a statement reminds one of a remark made by Aristotle, namely, that "nature makes so gradual a transition from the inanimate to the animate kingdom, that the boundary lines which separate them are indistinct and doubtful." Echoes of this statement are found in Boycott's address, made more than two thousand years later, before the Pathological Section of the Royal Society of Medicine; in 1928 he expressed the following opinion: "If you like to be paradoxical, you can say that live things are dead, or if you prefer it, that dead things are alive. Both at bottom have much the same characters, and it is unlikely that any sharp distinction between them can be drawn."

The nature of viruses interests scientists in general, but for physicians and public health officers there is an additional reason why viruses are of great interest and importance, *viz.*, the fact that they produce diseases many of which are epidemic in character. Furthermore, this fact was discovered without understanding the nature of the incitants, and the control of at least one virus malady, smallpox, was accomplished even before the relation of bacteria to disease was appreciated.

For many diseases induced by protozoa, fungi, bacteria, spirochetes, and rickettsiae methods of prevention or cure have been obtained. On the other hand, for a large number of maladies caused by viruses no method of prevention or cure has been found. Is this failure related to the nature of viruses? I doubt it. Most of the viruses that affect man seem to enter and leave their hosts through the upper respiratory tract. Consequently, none of the modern sanitary improvements, which have done so much to decrease certain kinds of disease, but which do not go so far as to sterilize the air breathed by human beings in their daily activities, has been effective in the control of many virus maladies.

As a result of recent comments on the origin and nature of viruses I have already heard it asked: "What is the use of attempting to prevent

the spread of virus diseases, if viruses are fabricated by their hosts?" Physicians, public health officers, and others interested in public health problems should not become alarmed or misled by questions or statements of such a character. In spite of a few unsubstantiated claims to the contrary, no instance of a virus disease arising *de novo* in a patient has been established. Furthermore, adequate control measures have already been found for some virus maladies, *e.g.*, smallpox and yellow fever, and there is every reason to believe that preventive or curative methods will be found to combat others. Indeed, all the evidence points to the fact that virus diseases, regardless of the nature of the active agent inducing them, will act in the future as they have in the past, that is, a patient who comes down with a virus ailment will do so because the initiation of the malady will have come about through the obtainment of the virus by the patient in some manner from without rather than through its fabrication *de novo* within. In other words, when one is developing plans for the control of virus maladies, there is as yet no reason to alter current ideas regarding the importance of obligate communicability of viruses in the origin and spread of virus infections.

In concluding, I desire to emphasize the following points. There is no adequate reason why inanimate agents cannot induce infectious diseases. Nor is it sensible to state that protozoa, fungi, bacteria, spirochetes, and rickettsiae are the only forms of living organisms capable of producing such maladies; we may be in the process of becoming aware of a new sort of living infectious agent. Thus, in regard to the nature of viruses, it seems that we are faced with three possibilities: Some of the viruses may be infinitely small living organisms, the midgets of the microbial world, possessed of a nature similar to that of living entities of sorts already known, ordinary bacteria and unicellular animals, differing from them only in respect to size; others may represent forms of life unfamiliar to us; while still others may be inanimate transmissible incitants of disease. One, two, or all three of the possibilities just mentioned may be found to hold. Consequently, final decision regarding the matter should be deferred. Statements made earlier in the paper must not be interpreted as denials of the *de novo* origin of infectious diseases in the past or of its possibility in the future. Such a denial would be foolish. Yet in spite of the fact that some infectious agents may turn out to be fabrications of their hosts, one can safely say that no one has yet established the *de novo* origin of a single infectious disease and that regarding such diseases the law of obligate communicability still prevails.

ISOLATION AND PROPERTIES OF TOBACCO MOSAIC AND OTHER VIRUS PROTEINS

Harvey Lecture, March 17, 1938

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IN ancient times disease was regarded as being due to supernatural agencies such as evil spirits and the will of the gods. Later the idea that disease resulted from natural causes such as comets, earthquakes, and the change of the seasons was quite widely held. About 100 B. C. Varro[1] and Columella[2] each expressed the idea that certain diseases might be caused by invisible living agents, but there was no experimental proof and the idea was not accepted. The writings of Fracastorius[3] about 1500 containing his theory of contagion, accurate descriptions of plague and of rabies, and a notation of the immunity that follows an attack of smallpox or of measles constituted an important advance, despite the fact that he made no special reference as to whether the contagious agents were living or non-living. It was not until about 1680 when, through the wonderful work of Leeuwenhoek,[4] the world of microscopic living organisms really became known. Although he described bacteria in 1683, it was over fifty years before his work was generally regarded as confirmed. Many workers considered these micro-organisms to be capable of causing disease, yet about a hundred years elapsed before their connection with disease was proved experimentally.

During the latter half of the nineteenth century there arose great controversies over the germ theory of disease, the nature of fermentation, and the age-old question of spontaneous generation, the latter of which had survived the blows administered by Redi[5] in 1668 and by Spallanzani[6] in 1776. These diverse yet related controversies were resolved through the brilliant researches of Pasteur,[7] Koch,[8] Tyndall,[9] Davaine[10] and others. It was proved for the first time that disease could be caused by a small living organism. The germ theory of disease emerged so triumphantly and was accepted so completely that thereafter there was a

definite tendency to regard all infectious diseases as being caused by bacteria. Thus, in 1892, when Iwanowski[11] discovered that the juice of a plant diseased with tobacco mosaic remained infectious after being passed through a filter which removed all of the known living organisms, he did not regard it as being especially significant and concluded that the disease was bacterial in nature. Six years later Beijerinck,[12] in a well planned and executed research, repeated and confirmed Iwanowski's experiments, and in addition demonstrated by serial passage of the filtrate that the disease was not due to a bacterial toxin. Beijerinck realized the significance of his results and referred to the infectious agent, not as being bacterial in nature, but as a contagious living fluid. Although he wished to differentiate it from ordinary bacteria, he too was thoroughly imbued with the idea that the infectious entity was living. These filtration experiments are regarded as the first demonstration of an agent that is now known as a virus. The same year Loeffler and Frosch[13] announced that the infectious agent of the foot-and-mouth disease of cattle would also pass through filters capable of retaining bacteria, and in 1901 similar results were obtained with the agent causing yellow fever in man.[14]

Characteristic Properties of Viruses

Since 1901 hundreds of the diseases of man, animals, and plants have been found to be caused by viruses. Included in this group are such diverse diseases as smallpox, rabies, psittacosis, fever blisters, epidemic encephalitis, yellow fever, poliomyelitis, fowl pox, hog cholera, dog distemper, equine encephalitis, certain types of tumorous growths in fowls and other animals, various yellows and mosaic diseases of plants, and possibly the transmissible lysis of bacteria. The earliest recognized property of the agents causing these diseases that was used to differentiate them from bacteria, namely, their filterability, has long since been generally recognized as untenable, for some of these viruses will not pass filters which will permit known organisms to pass. However, it has been replaced by certain other properties that are regarded today as characteristic of viruses. These emphasize the intimate relationship that exists between viruses and host cells, the fact that many virus-infected cells contain inclusion bodies, the fact that no virus has been grown on cell-free media, the fact that most but not all virus diseases are followed by a lasting immunity in recovered hosts, and the fact

that as a group viruses are smaller than ordinary bacteria. It should be emphasized that no single one of these properties may be used to differentiate viruses from bacteria and that, despite the attempted separation based on the properties just mentioned, viruses have nevertheless been generally considered as merely small ordinary living organisms, somewhat similar to the bacteria.

The fact that viruses may multiply or reproduce, that they may change or mutate and adapt themselves to new conditions, that they are specific in their action in that a given virus occurs or causes disease only in certain hosts, and that a lasting immunity follows most virus diseases has been used in arguments for the living nature of viruses, for these properties have been generally regarded as characteristic of living things. There were but few dissenters, and the large majority of the workers in the virus field saw no reason why viruses should not be considered small invisible living organisms. This conviction became even stronger with the discovery that some viruses were actually larger than certain bacteria.[15] However, in 1931 Galloway and Elford[16] reported that the virus of the foot-and-mouth disease of cattle was only about 8-12 mμ in diameter, only slightly larger than the familiar hemoglobin molecule and actually several times smaller than some of the hemocyanin protein molecules. Here, therefore, was a living organism that was smaller than a protein molecule! Evidence of a growing unrest and general dissatisfaction with this situation became noticeable in the writings of the time. Some of the virus workers realized the dilemma that had presented itself and attempted to find a solution. Thus, Burnet and Andrewes[17] in 1933 suggested that viruses might be divided into two groups, one consisting of organized living agents and the other of unorganized, supposedly non-living materials. Then they concluded that viruses affecting animals, presumably including the troublesome foot-and-mouth disease virus of molecular dimensions, were living organisms and belonged in the first group, whereas the bacteriophages ranging in size from about 10 mμ to 100 mμ and the large fowl tumor virus were placed in the second group. They apparently created the second group especially for the unusually small viruses and then neglected to use it for the foot-and-mouth disease virus. Rivers[18] was also troubled by the small size of this virus, and in his Harvey Lecture delivered four years ago suggested a division of viruses according to size. He considered that some might be minute living organisms, others representatives of a form of life unfamiliar to us, and still others non-living agents.

Selection of Tobacco Mosaic Virus

We thus have the unusual situation in which viruses, originally grouped together because of characteristic and similar properties, are subdivided solely on the basis of size, merely because it is repugnant to consider agents the size of protein molecules as living. A perusal of Figure 1, which shows the comparative sizes of entities ranging from the red blood cell, through bacteria and viruses, down to the egg albumin molecule, immediately reveals certain inherent difficulties in attempting such a subdivision. It may be seen that viruses form an unbroken series with respect to size from living organisms to protein molecules, and at either end there is an overlapping. Certain viruses are larger than accepted living organisms and other viruses are smaller than protein molecules. Where shall the lines subdividing the viruses be drawn?

I do not feel that we should permit ourselves to be drawn too far afield simply that we may preserve in our minds the supposed sanctity of the division between the living and the non-living. Let us, if necessary, revise our ideas and cease attempting to meet new situations with old definitions. So far as we know at the present time, viruses are similar in nature and there is no justification for attempting to subdivide them solely because of size. I consider, therefore, that the discussion this evening which is to center about tobacco mosaic virus is pertinent not only to this virus but to other viruses as well. Despite the fact that it affects only plants and is among the most stable of all viruses, it may be considered a representative virus with respect to the characteristic virus properties, and there is no reason to believe that knowledge gained through a study of tobacco mosaic virus may not, within certain limits, be applied to other viruses.

The unusual stability of tobacco mosaic virus has caused it to be an excellent subject for experimentation and as a consequence it has been used in numerous researches. One of the most extensive of the earlier studies was that of Allard[19] who, during the years 1916 to 1918, determined the effect of many different reagents on virus activity in an effort to learn something of the nature of the virus. In 1927 Vinson[20] undertook the purification of tobacco mosaic virus and with Petre reported in 1929 and 1931 on various procedures useful in separating the virus from much extraneous material. The crystalline material which was mentioned by Vinson and Petre in 1931 and which has been referred to editorially as

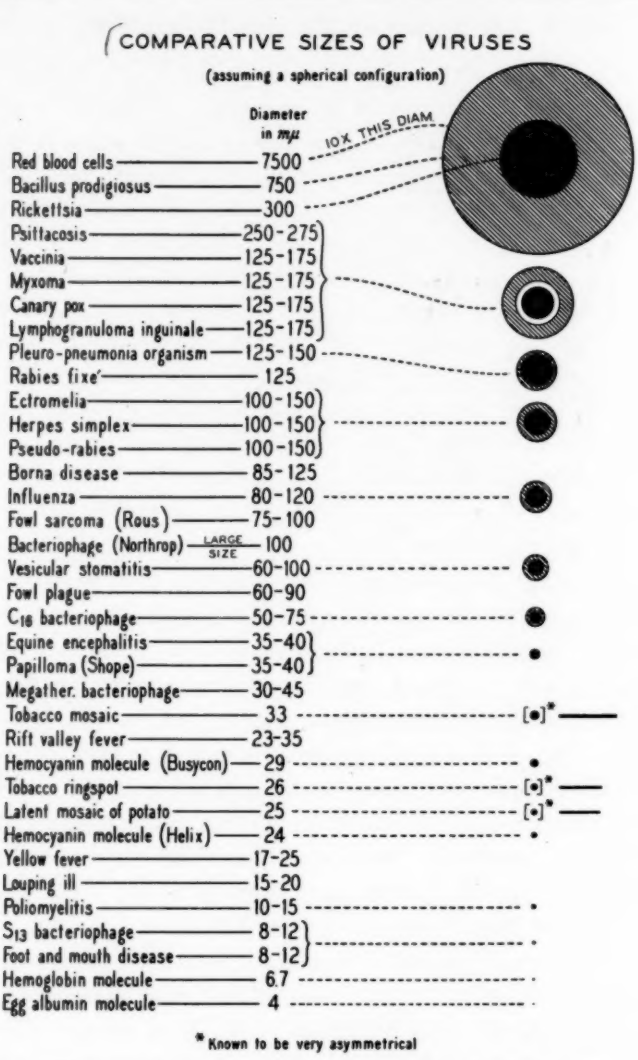
COMPARATIVE SIZES OF VIRUSES
(assuming a spherical configuration)
Diameter in mμ
10X THIS DIAM.
Red blood cells — 7500
Bacillus prodigiosus — 750
Rickettsia — 300
Psittacosis — 250-275
Vaccinia — 125-175
Myxoma — 125-175
Canary pox — 125-175
Lymphogranuloma inguinale — 125-175
Pleuro-pneumonia organism — 125-150
Rabies fixe — 125
Ectromelia — 100-150
Herpes simplex — 100-150
Pseudo-rabies — 100-150
Borna disease — 85-125
Influenza — 80-120
Fowl sarcoma (Rous) — 75-100
Bacteriophage (Northrop) — LARGE SIZE — 100
Vesicular stomatitis — 60-100
Fowl plague — 60-90
C18 bacteriophage — 50-75
Equine encephalitis — 35-40
Papilloma (Shope) — 35-40
Megather. bacteriophage — 30-45
Tobacco mosaic — 33
Rift valley fever — 23-35
Hemocyanin molecule (Busycon) — 29
Tobacco ringspot — 26
Latent mosaic of potato — 25
Hemocyanin molecule (Helix) — 24
Yellow fever — 17-25
Louping ill — 15-20
Poliomyelitis — 10-15
S13 bacteriophage — 8-12
Foot and mouth disease — 8-12
Hemoglobin molecule — 6.7
Egg albumin molecule — 4
* Known to be very asymmetrical

Fig. 1—A chart showing the relative sizes of several selected viruses including bacteriophages, as compared to those of the red blood cell, *Bacillus prodigiosus*, rickettsia, pleuropneumonia organism, and protein molecules. Three of the viruses have markedly asymmetrical configurations and are represented by the broad lines drawn to the right of the circles. Although these lines are drawn to represent rods having a circular cross section and a volume equivalent to that of the sphere, they should be regarded merely as illustrating markedly asymmetric particles. The figures used in the chart have been arbitrarily selected from the data of Elford, McIntosh, Bauer, Schlesinger, Svedberg, and others.

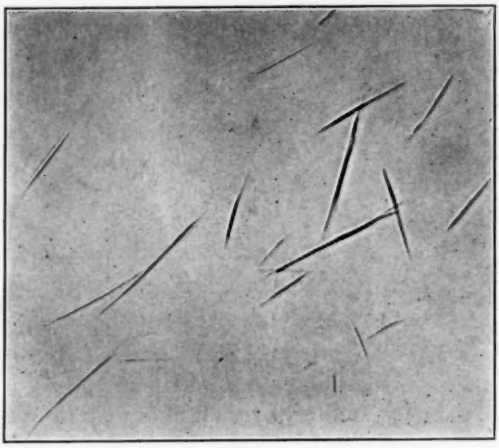

Fig. 2—Crystalline tobacco mosaic virus protein prepared by Dr. H. S. Loring. x 675 (Photo., J. A. Carlile).

crystalline virus actually consisted largely of inorganic matter having no connection with the virus. Vinson and Petre found the crystals to contain about 33 per cent ash and to lose activity on recrystallization, and they concluded that the crystals did not represent pure virus. The active crystalline material containing no demonstrable nitrogen, which was obtained by Barton-Wright and McBain[21] by means of Vinson and Petre's lead acetate method, was found by Caldwell[22] to consist of virus adsorbed on crystals of inorganic material.

Isolation and Properties of Tobacco Mosaic Virus Protein

Early in 1935 there was isolated for the first time an unusual, high molecular weight crystalline protein which possessed the properties of tobacco mosaic virus and which since has become known as tobacco mosaic virus protein.[23] The crystals of this protein are reproduced in Figure 2. This material was isolated by means of a chemical procedure that involved the use of one step of Vinson and Petre's lead acetate method but which was based chiefly on the general methods of protein chemistry that had been used so successfully by Northrop[24] and associates for the isolation of enzymes. The two properties of this protein that immediately set it apart from other proteins were that it carried high virus activity and that it had a molecular weight greater than that of any other known protein. One cc. of a solution containing only one part of

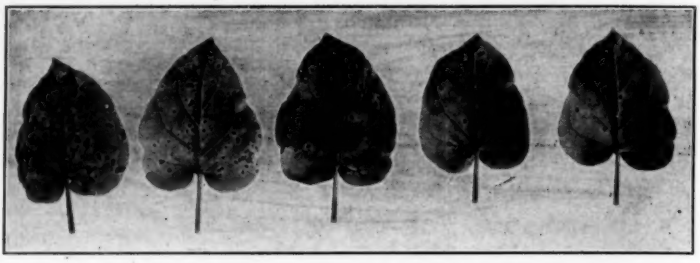

Fig. 3—Local lesions on leaves of plants of *Nicotiana glutinosa* showing effect of diluting juice containing tobacco mosaic virus (1:1, 1:3, 1:10, 1:100, and 1:1000). (From Holmes.[25])

this virus protein in ten billion parts of phosphate buffer was usually found infectious. The disease produced in plants by this as well as by more concentrated solutions was the typical tobacco mosaic disease, and from such plants more virus protein of the same kind as that introduced could be isolated. The activity of the virus protein may be determined with considerable accuracy by means of the Holmes[25] local lesion method. About forty-eight hours after a virus preparation is rubbed, by means of a bandage gauze pad, over the upper surfaces of the leaves of certain plants, necrotic lesions or spots appear. As may be seen in Figure 3, the number of lesions or spots may be used as an index of the amount of virus in the inoculum, for the more virus there is present the greater is the number of lesions that is obtained. When the method is suitably standardized by means of inoculating an unknown and control preparation on different portions of the same set of leaves, the virus activity may be determined with an error no greater than about 10 per cent.[26] It may be noted that Calmette and Guérin[27] in 1901 used the local lesion response as a measure of the potency of preparations of vaccinia virus.

Preliminary experiments on the diffusion and osmotic pressure of the virus protein indicated that it had a molecular weight of the order of several millions. Since these methods are not well suited for such huge molecules, a sample was sent to Dr. Svedberg for an ultracentrifugal analysis. The molecular weight based on a dissymmetry constant of 1.3 was found to be seventeen millions.[28*] The question that was immediately asked and that became of paramount importance was, "Is this

* Recent data indicates a dissymmetry constant of 2.52 which would give a molecular weight of about fifty millions. See: Lauffer, M. A. The molecular weight and shape of tobacco mosaic virus protein. *Science*, 1938, *84*:469.

unusual, high molecular weight protein tobacco mosaic virus?" On the present occasion I think that we may seek the answer to this question by means of the application of a chemical version of Koch's postulates. You are all familiar with the methods that are used by the bacteriologist to isolate and recognize a given organism. You are probably less familiar with the methods of the chemist, yet I can assure you that, because the compounds with which the chemist works have certain definite characteristic properties, they can be recognized with an accuracy that is no less than that involved in the bacteriologist's recognition of an organism. Tobacco mosaic virus protein has a definite and specific virus activity, chemical composition, x-ray diffraction pattern, ultraviolet light absorption spectrum, isoelectric point, sedimentation constant, diffusion constant, solubility, gives the usual protein color reactions, and is precipitated by the usual protein-precipitating agents.[23] In solution it has a characteristic opalescence, shows a marked Tyndall cone, exhibits strong double refraction of flow, and takes on a characteristic satin-like sheen when stirred. Concentrated solutions on standing form two definite layers that possess different physical properties. The protein has characteristic heat and pH stability ranges and is denatured only under certain definite conditions. Solutions containing but 10^{-7} gm. of the protein give a specific precipitin reaction with antiserum to the protein. These are some of the properties that are used to characterize the protein. So far as is known, this set of properties is not possessed by any other entity. The entire science of chemistry is built upon the recognition of substances by means of such properties, and I consider the recognition of tobacco mosaic virus protein by means of its properties to be as valid as the means used by the bacteriologist to identify a given organism.

Since there is no difficulty in recognizing the virus protein, we may proceed with our consideration from the standpoint of Koch's postulates. In the first place, this same protein possessing the same set of characteristic properties should be present in every case of the tobacco mosaic disease. During the past three years several hundred batches of mosaic-diseased Turkish tobacco plants were examined, and protein possessing identical physical, chemical, biological, and serological properties was obtained from all batches of plants worked up under comparable conditions. When different methods of purification were used, the protein was found to differ slightly depending upon the method used for isolation. However, in the case of this first postulate an even more severe

test may be applied, for tobacco mosaic virus has one of the widest host ranges known. It causes disease not only in several species of tobacco but also in plants such as spinach and phlox which are so distantly related that their normal constituents give no cross precipitin reaction with antiserum to the normal constituents of tobacco. Now, what do we find when we examine mosaic-diseased plants belonging to different species? The protein isolated from Burley tobacco, tomato, common nightshade, petunia, spinach, and phlox plants diseased with tobacco mosaic has been found to possess, so far as determined, the same physical, chemical, biological, and serological properties as those of the tobacco mosaic virus protein first isolated from diseased Turkish tobacco plants. The first postulate is fulfilled quite satisfactorily, therefore, since the virus protein has been found in every case of the tobacco mosaic disease.

Viruses have never been grown in the absence of cells, hence it is impossible to fulfill the second postulate as stated. However the third and fourth postulates are fulfilled quite readily, for I have already mentioned the fact that inoculation of any susceptible host with the virus protein results in the production of the typical tobacco mosaic disease and from these plants may then be isolated more of the same kind of protein as that used for inoculum. The successful application of Koch's postulates to tobacco mosaic virus depends, therefore, only upon whether or not means for satisfying the essence of the requirements of the second postulate can be found. The original purpose of this postulate was the demonstration, beyond a reasonable doubt, that the infectious agent could be obtained in pure form. It so happens that the question of the purity of the virus protein can be attacked best, not by cultivation methods, but by physical, chemical, and serological methods. However, if a material can be proved pure by these methods, I think that there is valid reason for considering the second postulate to have been fulfilled. From a chemist's standpoint, the question of purity is of the utmost importance, for if the infectious material is pure it follows directly that it is the virus. This question has, therefore, been studied at considerable length by means of as many different types of procedures as it has been possible to devise. The isolation of a protein having the same physical, chemical, biological, and serological properties from many different batches of diseased Turkish tobacco plants and from many other species of diseased plants is, of course, good presumptive evidence that a single substance is under consideration. There are, however, several more direct methods for deter-

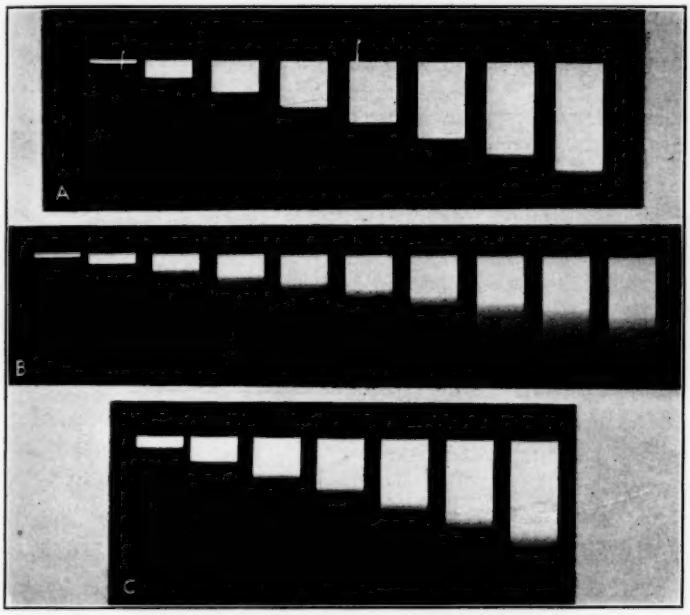

Fig. 4—Sedimentation pictures prepared by Dr. Wyckoff of solutions of tobacco mosaic virus protein. (a) Protein isolated by mild means such as by differential centrifugation, (b) following development of a second component caused by allowing protein to stand in the presence of salt, and (c) following more extensive treatment with salt.

mining the homogeneity of a preparation. Repeated crystallization with retention of constant properties has long been considered a criterion of purity. Despite the fact that this procedure has occasionally proved none too efficacious in the case of certain proteins, it seemed desirable to apply it to the virus protein. It was found that the virus activity of protein crystallized once was unchanged following fifteen successive crystallizations or following drastic fractional crystallization, provided the experiments were carried out rapidly in the cold and with low concentrations of salt. Although these experiments may not be considered conclusive proof that the virus protein is pure, they do demonstrate that it is impossible to detect an impurity by this method of fractionation. Eriksson-Quensel and Svedberg[28] found the protein to be completely homogeneous with respect to its electrochemical behavior. These workers

also made an ultracentrifugal analysis and found some of the earlier preparations to be somewhat inhomogeneous with respect to sedimentation constant. Wyckoff,[29] using some of our later preparations that were prepared by a less drastic method, found the protein to give the single sharp boundary shown in Figure 4a that is characteristic of a single molecular species. The protein forms a second component on standing in the presence of a little salt (Figure 4b), and on more extensive treatment becomes quite inhomogeneous (Figure 4c). The remarkable homogeneity of carefully prepared tobacco mosaic virus protein with respect to sedimentation constant and electrochemical behavior provides additional evidence for the purity of the protein.

The ultraviolet absorption spectrum of the virus protein was determined by Dr. Lavin[30] and found to have a maximum at about 2600-2650 A°. The absorption spectrum of the virus protein was found to agree essentially with the destruction spectrum of virus activity,[31] that is, just those wave lengths of ultraviolet light that were preferentially absorbed by the protein were exactly the same ones that caused inactivation. There is no doubt but that the light is absorbed by the protein, and the fact that this absorption of energy by the protein results in loss of virus activity is good evidence that the activity is a property of the protein. Further evidence relating activity and protein was obtained by denaturation of the virus protein by different methods. If the activity is a specific property of the protein, partial or complete destruction of the protein should result in a corresponding loss of virus activity. It was found that partial or complete denaturation of the protein by heating or by the use of acid, alkali, or chemical reagents was always accompanied by a corresponding loss of virus activity. For example, as may be seen in Figure 5, the sedimentation constant and virus activity of the protein remain unchanged following adjustment of solutions to hydrogen ion concentrations between about pH 2 and pH 8.[32,33] At more acid or alkaline reactions the virus activity is lost rapidly, and at exactly the same hydrogen ion concentration the protein is denatured and broken up into material having much lower sedimentation constants.

Correlation of Virus Activity with Protein

With the present method of inoculation, solutions containing from about one hundred to about one million molecules of the protein per cc. are required to cause infection. It may be argued, therefore, that the high

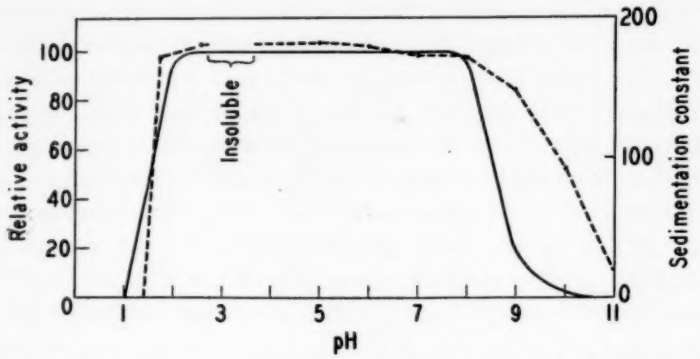

Fig. 5—pH stability range of tobacco mosaic virus protein as measured by virus activity (solid line) and by sedimentation constant (dotted line). (Drawn from data of Best and Samuel,[32] Stanley,[23] Eriksson-Quensel and Svedberg[28] and Wyckoff.[33])

molecular weight protein is not the virus and that the activity is due to virus adsorbed on the protein. It is unlikely that it will be possible to demonstrate experimentally whether or not one molecule of high molecular weight protein may cause infection until the method of inoculation is improved. However, there are other ways of approaching this question experimentally. The high molecular weight protein is isoelectric at about pH 3.5 and possesses a negative charge at more alkaline reactions and a positive charge at more acid reactions. It can be shown that, whether a hypothetical virus-carrying entity possess a constant negative charge, a constant positive charge, or be isoelectric at some hydrogen ion concentration other than that of the isoelectric point of the protein, it will possess the same charge as the high molecular weight protein at some reaction more acid or alkaline than pH 3.5. At the hydrogen ion concentration where the high molecular weight protein and the hypothetical virus entity possess the same charge, they should be separated from one another. Then, if the hypothetical entity is considerably larger or smaller than the high molecular weight virus protein, it should be possible to effect physical separation of the two by centrifugation. The virus protein is insoluble at its isoelectric point and may be readily sedimented to give a supernatant liquid that contains no protein and possesses no virus activity. Furthermore, as may be seen from Table 1, it was found experimentally that, when solutions of virus protein were centrifuged at pH 2.4 where the protein possesses a positive charge and at pH 6.7

where it possesses a negative charge, so that about 85 to 95 per cent of the protein was removed from the upper portions of the supernatant liquids, the virus activity of the separated upper and lower portions of the solutions was proportional to the amount of high molecular weight protein that they contained. These results prove that, when negatively charged protein ions, positively charged protein ions, or neutral protein are subjected to centrifugation, the virus and the protein sediment at exactly the same rate. These experiments provide, therefore, a very strong argument against the hypothesis that the virus activity is due to a separate entity adsorbed on the high molecular weight protein or,

TABLE I

Correlation of virus activity and protein on centrifugation of tobacco mosaic virus protein at pH 2.4 and 6.7[a].

pH during centrifugation	*Test No.*	*Protein concentration after centrifugation (mg. per cc.)*	*Portion of centrifuged sample used*	*Protein concentration used for tests (gm. per cc.)*	
				10^{-4}	10^{-5}
2.4	1	1.2	Upper portion	57.9[b]	25.3
		28.3	Lower portion	62.1	30.4
			No. of half leaves	52	52
			M.D./S.D.[c]	0.96	2.34
	2	1.2	Upper portion	58.1	30.6
		28.3	Lower portion	68.3	30.9
			No. of half leaves	56	56
			M.D./S.D.	2.51	0.15
6.7	1	1.2	Upper portion	145.0	74.8
		16.8	Lower portion	161.2	82.8
			No. of half leaves	56	56
			M.D./S.D.	2.02	1.89
	2	1.2	Upper portion	70.3	22.0
		16.8	Lower portion	79.9	25.8
			No. of half leaves	52	52
			M.D./S.D.	2.02	2.03

[a]Tests following dilution of lower portions to same protein concentration as in the corresponding upper portions of centrifuged samples. All dilutions were made with 0.1 M phosphate buffer at pH 7. *Phaseolus vulgaris* was used as the test plant.

[b]Numbers opposite a given preparation represent the average number of lesions per half leaf obtained on inoculation with the designated preparation and concentration. A given preparation was administered to the right halves of half of the leaves and to the left halves of the remaining leaves in each test.

[c]To show a significant difference between the mean number of lesions in any one experiment, the ratio of the mean difference (M.D.) to the standard error of the mean difference (S.D.) should not be less than 2.1.

for that matter, to a dissociable active group attached to the protein, and are direct evidence that the virus activity is a specific property of the protein.

Another method of approaching the question as to whether or not virus is merely adsorbed is to mix various proteins with the virus protein and determine whether or not the virus protein can be recovered with its characteristic virus activity. If virus were merely adsorbed on the high molecular weight protein, it seems likely that some of it would be lost and remain with the other proteins. However, it was found that from mixtures of virus protein with egg albumin, globin, trypsin, or pepsin it was always possible to recover the protein with its virus activity unchanged. Gratia and Manil,[34] working with mixtures of virus protein and phage protein, were also able to demonstrate that the two could be separated by centrifugation or by crystallization of the virus protein. They found, for example, that following four crystallizations of the virus protein the phage titer had dropped to 1/100 its original value. Basset, Gratia, and co-workers[35] also studied the effect of high pressure on virus activity, the ability to precipitate with antiserum, and the ability to crystallize, of purified virus protein. They found that these properties were unaffected by pressures up to about 6,000 atmospheres, but that at 8,000 atmospheres' pressure each of these properties was practically destroyed.

Double Refraction of Flow and Layering Phenomenon

There is another interesting and unusual property of the virus protein that may well be considered here, because it also results in a fractionation of the protein. Takahashi and Rawlins[36] noted in 1932 that the juice of mosaic-diseased plants was doubly refracting when made to flow, whereas the juice of normal plants failed to exhibit this phenomenon. Recently these workers also found that the suspensions and solutions of crystalline tobacco mosaic virus protein show double refraction of flow.[37] Evidence has been obtained by Bawden, Pirie, and co-workers[38] and in the writer's laboratory by Dr. Lauffer[39] that the molecules of virus protein are markedly asymmetric and have a length between ten and thirty times greater than their cross section. The rod-like shape of the virus protein molecules is apparently responsible for the separation of rather concentrated solutions of virus protein into two distinct layers, a phenomenon that was first reported by Bawden and Pirie.[38] As may be

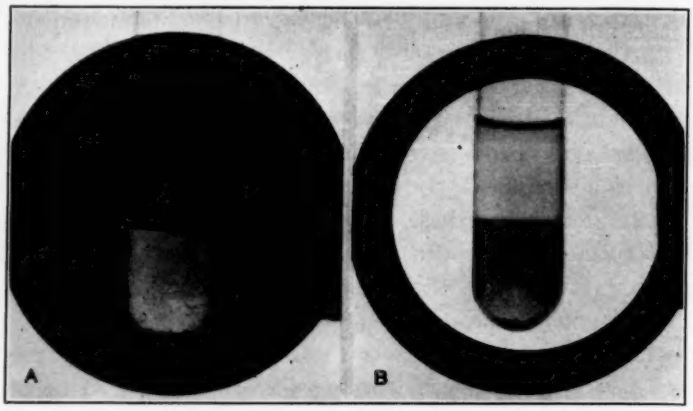

Fig. 6—Photograph in polarized light of a concentrated solution of tobacco mosaic virus protein that has been allowed to stand. (a)Test tube with crossed Polaroid plates on opposite sides. (b)Test tube with parallel Polaroid plates on opposite sides. The lower layer is spontaneously doubly refracting, whereas the upper layer is not. (Photograph by J. A. Carlile.)

seen from Figure 6, the line of demarcation between the layers is quite sharp. The upper layer shows double refraction only when made to flow and has a lower protein concentration than the lower layer. The latter is spontaneously doubly refracting apparently because, as the rod-shaped virus protein particles become sufficiently concentrated, they lose their ability to rotate about their shorter axes and become orientated. The lower layer appears to consist of a three dimensional mosaic of regions arranged at random to each other but in each of which all of the rod-shaped particles are orientated and are parallel to one another. Under the polarizing microscope the lower layer material when placed on a slide under a cover slip appears to consist of a two dimensional mosaic of doubly refracting areas orientated in different directions. The orientation phenomenon is readily reversible, for lower layer material may be diluted to give upper layer material and the latter may be concentrated to give lower layer material. However, it was of some interest to determine whether or not there was a difference in the virus activity of the two layers. This was done by Bawden and Pirie,[38] and the virus activity of the two layers was found to be exactly proportional to their protein content. Therefore, the fractionation of the protein that may be secured by virtue of this most unusual property also failed to reveal inhomogeneity.

Precipitin and Anaphylaxis Tests

There are still other properties of the virus protein, such as its immunological properties, that may be examined in connection with the question of homogeneity and that should be of especial interest on the present occasion. Purdy (Beale)[40] in 1928 and 1929 noted that the juices of mosaic-diseased plants contain an antigen specific for the virus-containing extracts and not present in the juices of normal plants. Beale,[41] Chester,[42] and other workers extended the serological work and found that antiserum to tobacco mosaic virus not only gave a precipitin reaction with extracts containing this virus and failed to give a precipitin reaction with extracts containing different viruses, but that it also possessed a specific neutralizing effect on tobacco mosaic virus. Despite the fact that no direct proof was available, it was generally considered that the antigen involved in these reactions was the virus itself. It is now known that the antigen was actually tobacco mosaic virus protein. Antiserum to purified virus protein gives a precipitin reaction with solutions containing but 10^{-7} gm. of virus protein per cc. and gives no precipitin reaction with extracts of normal plants or of plants diseased with other viruses. The precipitin reaction is generally regarded as a very sensitive test, and the fact that antiserum to virus protein reacts only with virus protein demonstrates that by this test the protein is homogeneous. It should be noted, however, that it is possible to inactivate the virus protein by very mild treatment such as irradiation with ultraviolet light or reaction with formaldehyde, nitrous acid, or hydrogen peroxide, without seriously altering the serological reactions.[43] Antisera to such inactive proteins give a precipitin reaction with either active or inactive protein and also have a specific neutralizing effect on virus activity. The latter fact is of considerable importance, not only because it may serve as an example of the immunological potentialities in the control of virus diseases, but also because it indicates a close relationship between virus activity and protein.

Another reaction that has been found even more sensitive than the precipitin test is that of anaphylaxis. Chester[44] in 1936, using the Schultz-Dale technique, found that the smooth muscle of the uteri of guinea pigs sensitized to some of the chemically prepared samples of virus protein reacted not only to virus protein but also to proteins extracted from normal plants, but that, following desensitization with proteins

from normal plants, no reaction was obtained with virus protein. These results indicated not only that these samples of virus protein contained a small amount of normal plant proteins as impurity but, more surprising, that the virus protein was not anaphylactogenic. It was found possible to remove the normal plant protein from the virus protein by sedimentation of the latter several times in a high-speed centrifuge or, as was done by Bawden and Pirie,[38] by digestion with trypsin, and to obtain virus protein that gave no cross reaction with normal plant protein. This purified protein was examined by means of the *in vitro* method and found to give no anaphylactic reaction. However, the same sample of virus protein was tested in sensitized guinea pigs *in vivo* by Seastone, Loring, and Chester[45] and found to be anaphylactogenic. These workers concluded that the distribution of the virus protein by the vascular system probably resulted in a more intimate contact with sensitized tissue than was possible in the isolated uterine horn, the exterior of which may be quite impervious to so large a protein molecule. It may be noted, however, that it is possible to prepare virus protein that even by the very sensitive precipitin and anaphylactic tests shows no evidence of containing impurities.

Partial Reactivation of Formolized Tobacco Mosaic Virus Protein

There is another type of experiment that I think should be considered in connection with the question as to whether or not the virus protein is homogeneous. If the protein molecule could be altered chemically with a simultaneous change or loss of virus activity and the molecule subsequently returned to its original structure with a simultaneous return of virus activity, it would serve as strong evidence that the activity is a specific property of the protein. There have been several reports on the reactivation of viruses. Zinsser and Seastone[46] noted in 1930 that occasionally they were able to reactivate, by means of the reducing agent cysteine, preparations of herpes virus that had been inactivated presumably by mild oxidation resulting from exposure to air. Similar results by means of reduction have been reported with this and other viruses by different workers. Vinson and Petre[20] found that safranine or lead subacetate precipitated tobacco mosaic virus in the form of insoluble complexes possessing practically no virus activity, but that by removal of the precipitating agents most of the original activity could be regained. These results have been confirmed by the writer who found in addition

that silver nitrate formed a silver salt of the virus protein that possessed no virus activity, but which could be reactivated by removal of the silver ions by dialysis. However, in all of this work no attempt was made to correlate the structure of the protein with virus activity. It was not even demonstrated that the inactivation caused by safranine, silver, or lead salts was real and not due to insolubility or to toxicity.

Recently, Dr. Ross,[47] working with tobacco mosaic virus protein in the writer's laboratory, completed a research in which not only true inactivation and reactivation of tobacco mosaic virus was demonstrated, but also in which changes in the structure of the protein molecule were followed by chemical means. It was found that the inactivation of virus protein by formaldehyde followed roughly that of a monomolecular reaction and was accompanied by a decrease in amino nitrogen as measured colorimetrically or by means of the Van Slyke gasometric method and by a decrease in the color developed by Folin's tyrosine reagent. The inactivation is not due to the formation of an insoluble or toxic compound, for the inactivated protein was found to be soluble and to be no more toxic than egg albumin or hydrogen peroxide-inactivated virus protein. The inactivation is not due to the presence of free formaldehyde, for the concentration of free formaldehyde was proved to be less than 10^{-5} gm. per cc., a concentration which was found to have no effect on virus activity. When the inactivation reaction was stopped after suitable periods of time by dialysis at pH 7 to remove the excess formaldehyde, it was possible to obtain partially or completely inactivated virus protein that could be reactivated to a marked extent by dialysis at pH 3. The reactivation was accompanied by an increase in amino nitrogen as measured by the color developed with ninhydrin and by an increase in the color developed with Folin's tyrosine reagent. Preparations completely inactive when inoculated at a concentration of one mg. of protein per cc. were found to possess a definitely measurable amount of virus activity following reactivation. Preparations containing 0.1 and 1 per cent, respectively, of the original activity were found following reactivation to contain about 1 and 10 per cent, respectively, of the original virus activity. In other words, it was possible to obtain a 10-fold increase in virus activity by means of the reactivation technique and to demonstrate a simultaneous change in the structure of the protein molecule. Although the exact structural changes have not been determined as yet, it has been possible to measure them, and I feel that it is

highly unlikely that these changes that have been measured are merely fortuitous and have nothing to do with activity. I consider that the reactivation of formolized virus protein brings strong evidence that the virus activity is a specific property of the protein and provides some information relating the structure of the protein to virus activity.

Summary and Discussion of Evidence Relating to Tobacco Mosaic Virus Protein

Now let us consider the available evidence pertinent to the question as to whether or not tobacco mosaic virus protein is pure and hence is the virus. The virus protein isolated from many different batches of diseased Turkish tobacco plants or from other plant species diseased with the mosaic virus was found to possess the same chemical, physical, biological, and immunological properties, and these properties were found to remain unchanged following fractionation of the protein by various procedures. The protein was found to be completely homogeneous with respect to its sedimentation constant and electrochemical behavior. It was found impossible to separate the virus activity from the protein by any one of several procedures. The absorption spectrum of the protein was found to agree essentially with the destruction spectrum of virus activity. It was found impossible to demonstrate the presence of an impurity in purified preparations of virus protein even by the sensitive precipitin and anaphylactic reactions. The pH stability range of the protein was found to coincide exactly with that of the virus activity. Partial or complete denaturation of a protein preparation by any one of several procedures was always found to result in a corresponding loss of virus activity. Finally, it was found possible not only to inactivate and reactivate the virus protein, but also to demonstrate that the inactivation and reactivation were accompanied by simultaneous changes in the structure of the protein molecule. Thus, by all of the tests that it has been possible to devise, the virus protein is homogeneous. I should hesitate to conclude as a result of any one test that a material is homogeneous and hence pure. However, when a material is found homogeneous by several quite different types of tests, I consider it highly significant. The various tests for homogeneity that have been applied to the virus protein are as valid as the cultural and microscopic tests used by the bacteriologist, hence it may be concluded that the essence of Koch's second postulate has been fulfilled. In addition, considerable

evidence of a more or less direct nature that the virus activity is a specific property of the protein has been obtained. In all of the work that has been done on tobacco mosaic virus protein in the writer's laboratory and that has been reported from other laboratories, not one bit of evidence that the purified virus protein is inhomogeneous or that the virus activity is due to material other than the high molecular weight protein has been obtained. If a decision be required at the present time, it is impossible from a chemist's standpoint or as a result of the application of Koch's postulates to conclude other than that the virus protein is actually tobacco mosaic virus.

However, just as the possibility exists that tuberculosis may some day be found to be due not to the tubercle bacillus but to something adsorbed on the organism, the possibility exists that the virus activity may be due not to the protein but to an impurity adsorbed on the protein that cannot be detected by means now at our disposal, but which may some day be detected by more refined methods. It is impossible to obtain final conclusive proof that any given material is pure, and the possibility that a certain property of any given material may be due to an impurity must always remain, regardless of the material. Insulin was discovered seventeen years ago, was crystallized twelve years ago, and has been subjected to extensive investigation. Although the biological activity of insulin is generally regarded as a property of the protein, there are workers who feel that the activity may be due to a dissociable group attached to the protein or to a separate entity adsorbed on the protein, and that eventually it may prove possible to separate the active agent from the protein. Should this ever prove possible or should it ever prove possible to separate the virus activity from the protein, I should regard it not as a catastrophe but as a most important and welcome advance. We would then be able to throw away 99.9 per cent of the virus protein or of the insulin protein and in the small remaining fraction we would retain all of the original activity. Now, it should be noted that by virtue of the experimental evidence that has already been accumulated with respect to these two proteins, the impurities or active agents could hardly be other than closely related proteins, hence it would still follow that the virus and insulin are proteins, but possessing activities a thousand or more times greater than the materials now known. According to present standards, such materials would be most amazing agents and their isolation would constitute a most important discovery. Although we

should recognize this possibility that the virus activity may eventually be found not a property of the high molecular weight protein, I think we should also recognize the fact that there is no reason to believe that such a situation actually prevails and that, according to all of the evidence available at present, we may conclude that the virus protein is actually the virus.

Isolation of the Virus Proteins of the Strains of Tobacco Mosaic Virus and of Other Viruses

Because of the life-like properties that are characteristic of viruses, the conclusion that the high molecular weight protein is the mosaic virus is fraught with implications of importance. Before discussing these, however, I should like to consider whether or not the general conclusion can be justified, firstly, with respect to the strains of tobacco mosaic virus and, secondly, with respect to different viruses. There is good evidence[48,49] that as ordinary tobacco mosaic virus multiplies within a host it occasionally mutates or in some manner becomes altered so that new and different strains of virus arise. These strains may be separated and isolated by means of an appropriate technique[48,50] and, although some occasionally revert to the ordinary strain, there is a definite tendency for them to remain as distinct strains of the mosaic virus. There are, therefore, several well recognized strains of tobacco mosaic virus. It was of considerable importance to determine whether or not plants diseased with strains of tobacco mosaic virus would contain high molecular weight proteins and, if so, whether or not these proteins would be similar to tobacco mosaic virus protein. The problem has been studied in the writer's laboratory and by Bawden and Pirie[38,51] in England. It was found that from plants diseased with strains of tobacco mosaic virus such as aucuba mosaic, enation mosaic, and the Holmes masked strain could be isolated high molecular weight virus proteins that were remarkably similar to tobacco mosaic virus protein but that differed in certain respects not only from each other but from the mosaic virus protein. For example, although the virus proteins of tobacco mosaic virus and its strains were found to have the same elementary chemical composition, optical rotation, crystalline appearance, and similar x-ray diffraction patterns and serological properties, it was possible to distinguish them by means of solubility and isoelectric point determinations, their reactions with clupein sulphate, and serologically by means of the

cross absorption technique.[23,38,51] The various strains of tobacco mosaic virus are characterized, therefore, by different although closely related high molecular weight proteins. Thus, when tobacco mosaic virus mutates or in some way becomes altered so that a new strain arises, this change is accompanied by the production of a new and slightly different virus protein. Inoculation of this new virus protein to susceptible hosts results not in the production of the ordinary disease and virus protein, but in the production of the new disease and of a virus protein of the same kind as that used as inoculum. This is exactly what would be expected to happen if the protein is the virus, and the fact that it actually happens serves as additional justification for the original conclusion that the protein is the virus.

Now let us examine the situation with respect to other viruses for, if tobacco mosaic virus is truly representative, it should be possible to isolate other viruses in the form of high molecular weight proteins. However, when the chemical method used for the isolation of the virus proteins of tobacco mosaic and its strains was first applied to plants affected by some of the less stable viruses such as those causing the tobacco ring spot, latent mosaic of potato, and severe etch diseases, it was not found possible to isolate high molecular weight proteins from such plants by this chemical method. These viruses are considerably less stable than tobacco mosaic virus and there was some indication that they existed in low concentration in the host. It seemed possible, therefore, that the chemical method might cause inactivation of these viruses due to their instability or that the method might not be sufficiently specific to separate a small amount of virus protein from a large excess of other proteins. The results demonstrated that the chemical method would have to be improved or a new method evolved, in order to work successfully with such viruses. Fortunately, about this time the development of the air-driven centrifuge reached a stage where it was possible to subject a hundred or more cc. of solution to high-speed centrifugation. In cooperation with Dr. Wyckoff,[52] it was found possible to isolate tobacco mosaic virus protein from the juice of diseased plants by means of differential high-speed centrifugation. The general method of differential centrifugation is not new, for it was used as early as 1922 by MacCallum and Oppenheimer[53] in work with vaccinia. It was used subsequently by Ledingham,[54] Craigie,[55] and Rivers[56] for the isolation and purification of the elementary bodies of vaccinia. Although high-

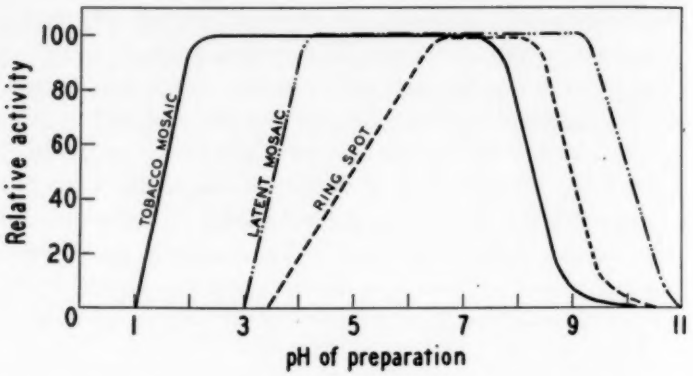

Fig. 7—pH stability range of activity of tobacco mosaic, tobacco ring spot, and latent mosaic of potato virus proteins. (Drawn from data of Best and Samuel,[32] Stanley[23,61] and Loring.[59])

speed centrifugation was used by Bauer and Pickels[57] to concentrate yellow fever virus, none of the smaller viruses had been isolated by the centrifugal method prior to 1936. Because of the ease and rapidity with which it was found possible to isolate tobacco mosaic virus protein by differential high-speed centrifugation, it seemed likely that the method should prove useful in the isolation of the less stable viruses.

In collaboration with Dr. Wyckoff,[58] batches of Turkish tobacco plants diseased respectively with tobacco ring spot, latent mosaic of potato, severe etch, and cucumber mosaic viruses were examined, and in every instance it was found possible to demonstrate the presence of a high molecular weight protein. Tobacco ring spot and latent mosaic virus proteins were isolated in sufficient quantity so that it was possible to study their physical, chemical, and serological properties. These properties were found to differ markedly not only from those of tobacco mosaic virus protein but also from each other. For example, ring spot virus protein causes the ring spot disease, appears homogeneous when examined in the ultracentrifuge, has a sedimentation constant of $S_{20^\circ} = 115$, and is completely denatured and inactivated after standing for one hour at pH 3 or following a five minute exposure to a temperature of 64° C. This virus protein was found to be about 10,000 times more active than the infectious juice used as starting material. Dr. Loring[59] has found the latent mosaic virus protein to have its own definite and highly

characteristic set of properties. Bawden and Pirie[51] have isolated two stable strains of cucumber mosaic virus in the form of crystallizable high molecular weight proteins by chemical means. They have just recently isolated[60] from tomato plants diseased with bushy stunt another virus protein that differs from those previously described, in that it crystallizes in the form of dodecahedra. It should be emphasized that these proteins differ markedly in their physical, chemical, biological, and serological properties. For example, in Figure 7 is given the pH range of stability of three virus proteins. Tobacco mosaic virus protein is native and active between pH 2 and 8, latent mosaic virus protein between pH 4 and 9.3, and ring spot virus protein between pH 6 and 8. At more acid or alkaline reactions than those given for each of these virus proteins, they disintegrate, become denatured, and lose their virus activity.

Another point that should be emphasized is that the concentration reached by these several virus proteins in the same host differs markedly. In Figure 8 are given the amounts of virus protein that were isolated from 200 gm. of tissue diseased with different strains of tobacco mosaic virus and with other viruses.[61] It may be seen that in the same host, Turkish tobacco, the concentration level reached by tobacco mosaic virus protein is greater than that of its two strains, aucuba mosaic and the masked strain, is 268 times greater than that of tobacco ring spot virus protein, and is 2000 times greater than that of cucumber mosaic virus protein. It may be noted that in the case of the tobacco mosaic disease in Turkish tobacco plants as much as 80 per cent of the total protein of the plant has been isolated in the form of the virus protein. Another fact of importance is that the same virus protein reaches a different level of concentration in different hosts. Thus, the level reached by tobacco mosaic virus protein in Turkish tobacco is 400 mg., in tomato 260 mg., and in spinach 30 mg. per 200 gm. of plant material. There is also included in this figure the amount of the homogeneous heavy protein carrying virus activity that Beard and Wyckoff[62] were able to isolate by means of differential high-speed centrifugation from 200 gm. lots of the warty tissue of rabbits diseased with the Shope papilloma virus. The concentration of this material is of the same order as that of some of the less abundant plant virus proteins. This material, that of the Rous sarcoma virus isolated by Claude,[63] the material containing a nucleoprotein isolated by Janssen[64] from tissue infected with the foot-and-mouth disease virus and the elementary bodies of vaccinia are now

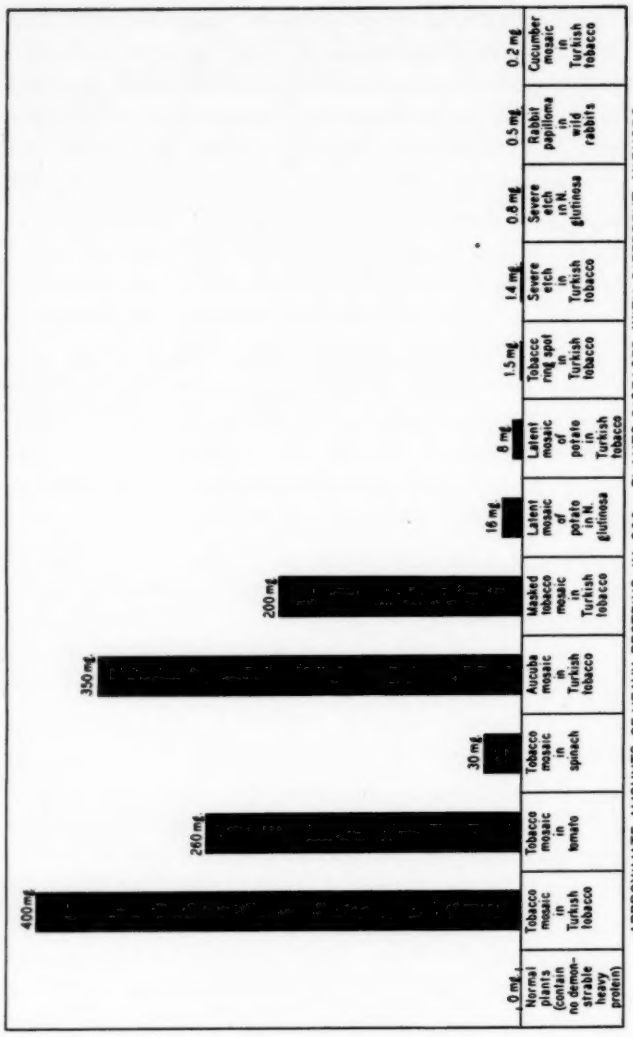
APPROXIMATE AMOUNTS OF HEAVY PROTEINS IN 200 g. PLANTS DISEASED WITH DIFFERENT VIRUSES
(The amount of the relatively abundant papilloma virus protein per 200 g. of whole rabbit is also cited
The protein is concentrated, however, in tissue representing about 1/60th of the whole rabbit)
0 mg.
Normal plants (contain no demonstrable heavy protein)
400 mg.
Tobacco mosaic in Turkish tobacco
260 mg.
Tobacco mosaic in tomato
30 mg.
Tobacco mosaic in spinach
350 mg.
Aucuba mosaic in Turkish tobacco
200 mg.
Masked tobacco mosaic in Turkish tobacco
16 mg.
Latent mosaic of potato in N. glutinosa
8 mg.
Latent mosaic of potato in Turkish tobacco
1.5 mg.
Tobacco ring spot in Turkish tobacco
1.4 mg.
Severe etch in Turkish tobacco
0.8 mg.
Severe etch in N. glutinosa
0.5 mg.
Rabbit papilloma in wild rabbits
0.2 mg.
Cucumber mosaic in Turkish tobacco

Fig. 8—Approximate amounts of heavy proteins in 200 gm. of tissue diseased with different viruses.

obtainable in reasonably large amounts. It seems likely that it will not be long before it will be possible to make a decision as to whether or not these materials are similar to the plant virus proteins. We may conclude from the amounts of the different plant virus proteins that have been isolated that a given virus may reach different concentration levels in different hosts and that in the same host different concentration levels are reached by different strains of the same virus and by different viruses. The amount of virus protein produced in a host is therefore dependent not only upon the virus but also upon the host.

Recovery of Tobacco Ring Spot Diseased Plants

In this connection I think that I should mention the interesting recovery phenomenon exhibited by Turkish tobacco plants diseased with tobacco ring spot virus. Following the initial violent attack, these plants appear to recover and present a normal appearance. This phenomenon was described by Price,[65] who showed that such recovered, apparently normal plants still retained virus, although in a reduced amount, and that they were immune and could not be reinfected. These recovered plants have now been examined and from such plants there has been isolated a virus protein that appears to be identical in all respects with that isolated from badly diseased plants. As was expected in view of Price's work, the amount of the protein in recovered plants was found to be only about one-sixth that in badly diseased plants. Recovery, therefore, appears to consist of some mechanism by means of which the amount of virus is reduced in concentration to a level that no longer causes disease symptoms. I have mentioned this phenomenon because it is a striking demonstration of the persistence of virus in recovered hosts, because it shows the effect of different levels of virus concentration in the same host, and because additional evidence correlating protein with virus was secured by the isolation of the same virus protein from apparently normal although immune plants.

Discussion

The isolation of several virus proteins from tissues diseased with different viruses and the demonstration that these virus proteins possess highly characteristic physical, chemical, biological, and serological properties that differ not only from each other but also from those of tobacco mosaic virus protein serve as additional justification for relating virus

activity to protein. We see, therefore, that all of the information that is now available regarding the homogeneity of the virus proteins, the relationship of virus activity to protein, the isolation of different strains of the same virus in the form of different although closely related proteins, and the isolation of different viruses in the form of quite different and highly characteristic proteins, indicates that the virus proteins are in fact the viruses themselves and that if a decision be required at present the only conclusion that is possible, based on experimental evidence, is that these proteins are the viruses.

Nevertheless, some workers refuse to entertain the idea that the protein may be the virus, because they dislike to consider that a protein molecule may possess certain properties such as the ability to reproduce and to mutate, properties that they like to consider as characteristic of living things. However, it is foolish to disregard experimental facts and to attempt to come to a decision solely on the basis as to whether a given substance does or does not possess properties characteristic of living things, for there is not a single property that has been considered characteristic of living things that may not be duplicated in a recognized non-living system. I do not mean by this that living things do not differ from non-living things. I do mean, however, that I feel we should not be too strongly influenced by the conventional criteria of life. As we go from the admittedly non-living to the admittedly living, I think that there must be a transition stage where there are entities that may possess some properties that are considered characteristic of living things and some properties that are considered characteristic of non-living things. What could fill this place more simply and logically than the high molecular weight virus proteins that are intermediate in complexity between the protein enzymes and hormones, the wonderful properties of which we already recognize, and the system of proteins that we call protoplasm and that constitutes life. There is evidence that even within the virus group there is a gradual increase in complexity of structure from the small nucleoproteins to the more elaborate elementary-body type of virus. There is, however, no sharp break despite the fact that in certain respects the structure of the latter may resemble that of a cell-type organism as much as it resembles that of the smaller viruses. I consider it unimportant whether we call the virus proteins molecules or organisms and this evening I have referred to them as molecules solely because of the accident of my training as a chemist. However, I consider

the correct recognition of their fundamental properties a matter of extreme importance.

I should like to point out that, although the endowment of a protein molecule with virus properties marks a new and previously unrecognized property of proteins, it cannot, in view of the unusual properties of the protein enzymes and protein hormones that have been recognized in recent years, be regarded as a totally unexpected property. Furthermore, although the recognition may constitute an advance, it certainly is not particularly enlightening, for in placing the secret of viruses within a protein molecule Nature has selected the least understood and the most mysterious of all the compounds with which the chemist works, for the structure of not one protein is known. In recent years the protein molecule has attracted the interest of workers in different fields and this community of interest is especially noteworthy at the present time in the case of the virus proteins. They interest the pathologist since they cause disease, and the bacteriologist because of their small size and because they possess certain properties that have been regarded as belonging to organisms. The chemist is attracted to them because, although they have many of the properties of molecules, they possess in addition properties that have not hitherto been ascribed to molecules. The physicist is interested in them because of their properties as macromolecules and because some virus proteins show that interesting layering phenomenon that has been called a new property of matter. They are of interest to the biologist because they possess properties that have been regarded as characteristic of both living and non-living things. The geneticist is interested in them because they undergo a phenomenon similar to mutation and thus may possibly permit a study of the nature of mutation from a new viewpoint. Lastly, they interest the philosopher because they permit him to enter with renewed vigor upon a discussion of that age-old question of "What is life?" The virus proteins thus bid fair to become the common meeting ground of scientists. The advance so far has been merely a more exact definition of the problem that confronts us, the problem of the protein molecule. The most interesting and important advances and at least one fundamental discovery lie ahead and depend upon the ability of workers in different fields of science to explore successfully the protein molecule.

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THE VASCULAR AND RENAL COMPLICATIONS OF PREGNANCY*

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UNTIL RECENTLY study of the so-called toxemias of pregnancy has been confined to the obstetric clinic largely to the exclusion of the internist and, too often, the pathologist. Of late, however, to the credit of a few obstetricians of vision, there is general realization of the fact that these disorders are quite as much the concern of general medicine as of obstetrics. The results of an increasing interest in and opportunity for medical study are now appearing. Notably studies from the Sloane Hospital for Women in New York, the New Haven Hospital and the Boston Lying-In Hospital have advanced our knowledge of the relation of these obstetric problems to those of general medicine.

From this pioneer work it seems clear that in the so-called toxic disorders of pregnancy we are not dealing with conditions of an isolated nature but with those having very definite relation to well recognized, if not well understood, disturbances common in the general medical clinic.

After all, toxemia of pregnancy is a general term under which are included a variety of deviations of the pregnant woman from the normal. In addition to their most common association with cardiovascular disease and with nephritis one may find less frequent complications included. These may be pernicious vomiting, atrophy of the liver, pyelonephritis, anemias or the psychoses. Such a loosely used and inclusive term as toxemia of pregnancy can no longer be accepted as precise or specific. It should be subjected to critical analysis, perhaps dissected until there is nothing left.

The student of the medical complications of pregnancy is impressed constantly by the rôle of pregnancy as an activator of latent disease. Repeatedly one sees tendencies to disorders, whether of a metabolic, infectious or psychic sort, develop during gestation into the fully drawn

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picture of disease. Perhaps this emphasizes a viewpoint at variance with the tradition that the toxemias are *sui generis* and peculiar to pregnancy and places a burden of proof upon one presenting the newer aspect.

In this paper discussion is limited to the vascular and renal disorders of pregnancy. It, therefore, may be well at the outset to give an accepted classification of these diseases.

Classification of Renal and Cardiovascular Diseases

1. *The inflammatory nephropathies—nephritis:*

GLOMERULONEPHRITIS
- diffuse
  - acute
  - chronic
- focal

PYELONEPHRITIS
- acute
- chronic

2. *The degenerative nephropathies—nephrosis:*

- chemical poisoning
- bacterial toxins
- circulatory disturbances
- special types
- amyloid
- lipoid

3. *Hypertensive cardiovascular disease, essential hypertension, hyperpiesis:*

SLOWLY PROGRESSIVE (BENIGN) FORM
Death from:
- apoplexy
- cardiac failure
- arteriosclerotic nephropathy with uremia

RAPIDLY PROGRESSIVE (MALIGNANT) FORM
Death from:
- necrotizing arteriolitis
- uremia

Nephritis Complicating Pregnancy

There has been much confusion in the use of the term nephritis. Many obstetric writers do not yet differentiate nephritis and vascular disease. This has given rise to needless argument and a confusion which lessens the value of much of the older writing upon the subject. If we are

strict in our definition and confine the term nephritis to the inflammatory states of the kidney as differentiated from the degenerative, we are on safer ground. Nephritis usually implies infection, ordinarily but not always with the streptococcus. The pneumococcus, staphylococcus or other organisms may cause renal inflammation. Certain chemical irritants as mercury; possibly, but, by no means surely, certain unidentified products of metabolism may give rise to renal inflammation not unlike that of the toxins of bacteria. Usually history is given of scarlatina, of tonsillar or other infections prone to involve the kidney secondarily. The consequences of these may not have been manifest prior to the pregnancy. It is a general principle that any organ performs its functions satisfactorily until its factor of safety is abolished. This is particularly true of the kidney. The demands of the fetus seem to trespass upon the renal reserve in proportion to the size or age of the product of conception. In consequence the evidences of renal breakdown are not likely to appear until pregnancy is well advanced, usually to the third trimester. In some cases in which a manifest nephritis is present before pregnancy, the symptoms become aggravated in the early phases of gestation.

Whatever the period of pregnancy at which symptoms appear, the earliest feature is the albuminuria which may vary from a slight trace to a urine that "boils solid" and may contain as much as 90 mg. of albumen for each 100 cc. of urine. Unless secondary to an intervening acute infection, albuminuria, once established, though variable in degree, is not likely to clear up so long as pregnancy lasts. Red blood cells, casts and renal epithelium are common urinary findings. In the more advanced stages the kidney becomes unable to excrete concentrated urine and the specific gravity may not rise above 1.010 under conditions of fluid deprivation. The excretion of phenosulphonephthalein is reduced. In general this test is unreliable in pregnancy as the readings in normal pregnant women are frequently below the expected average. Of the chemical findings in the circulating blood, the urea or urea nitrogen estimations are of greatest value in the differentiation of nephritis from vascular disease, since in the latter there is generally no retention of nitrogen until the end stages of the disease. The urea clearance test may give data of value. It seems to have about the same worth in the nephritis of the pregnant as in that of the non-pregnant.

Among the symptoms of nephritis most prominent are headache, nausea, vomiting, edema, secondary anemia, and visual disturbances some

of which may be consequent upon albuminuric retinitis. Cardiovascular disturbances seem to be secondary to the renal dysfunction and almost always are preceded by the typical urinary findings. While arterial hypertension is generally present, it may be absent. In advanced cases it may reach high figures. Both systolic and diastolic pressures are affected. In long standing cases there is cardiac hypertrophy with accentuated second sounds and perhaps a systolic murmur of hemic origin if anemia is pronounced or if a relative insufficiency of the mitral valve is present. A terminal event may be a pulmonary edema from left ventricular failure during or after a convulsion or labor. The peripheral vascular lesions are most variable. Excepting in long standing cases the larger arteries show little change. The ophthalmoscope may reveal nothing significant even in cases with an extreme degree of nitrogen retention. Again, one may see arterial spasticity, arteriovenous constriction, edema, even to the extent of separation of the retina; varying amounts of exudate and hemorrhages and the typical patterns of albuminuric retinitis. When well-marked retinal edema exists with hypertension, nitrogen retention and hyperreflexia, uremic convulsions may be anticipated.

The Effect of Nephritis upon the Course of Pregnancy and upon the Fetus

This is usually a problem of the later months of pregnancy. Nephritis is the most common cause of fetal death at this period of gestation. Evidence that this is the consequence of infarction of the placenta largely the result of arterial changes is not conclusive. Whenever albuminuric retinitis or nitrogen retention or oliguria or albuminuria are present and tend to increase, speedy fetal death may be predicted and can be prevented only by prompt emptying of the uterus. Sharp change in the fetal heart rate or enfeeblement of the heart sounds presage intrauterine death. Labor is premature, the fetus being expelled promptly or considerably later in a macerated state. If a living infant is born it faces the handicaps of prematurity and sometimes of toxic effects secondary to the maternal nephritis.

The effect of pregnancy upon the nephritic woman is most unfortunate. During the last trimester she is beset by the dangers of uremia, cardiac failure, apoplexy, serious impairment of vision, and inability to cope with infections. Even if she carries on to a successful issue, the pregnancy has caused a serious decline in the margin of renal safety and a

shortened life expectancy. Should another pregnancy occur renal breakdown will take place earlier and be more severe. Nothing is more unprofitable or dangerous than repeated attempts at childbearing on the part of the nephritic woman. After the pregnancy is over the clinical and laboratory evidences of the disease usually persist. In some of the milder forms they disappear and the disease again becomes latent.

In summary it may be said that nephritis complicating pregnancy (nephritic toxemia) is relatively rare, being from 5 to 10 per cent of all cases of late toxemia. Previous nephritis may have been latent or manifest, the former brought to light by the added strain of pregnancy. Excepting lues no malady so threatens the life of the fetus. The dangers to the mother are an aggravation of the nephritis including uremia, circulatory failure, and superadded infection. Repeated pregnancies are increasingly disastrous to both mother and child. Prevention of future pregnancies in the woman with established nephritis is obligatory. This may be accomplished by division of the tubes, by the Roentgen rays, by vasectomy in the husband or by the use of contraceptives.

Pyelitis and Pyelonephritis Complicating Pregnancy

Probably owing to the hydronephrosis and ureteral dilatation so common in pregnancy infection of the pelvis of the kidney is among the most frequent complications of that state. If it is neglected serious results may follow. Infrequently the acute inflammation may invade the renal parenchyma. In one type of such invasion the tubules and glomeruli may be infiltrated diffusely with bacteria and pus cells; in another, the interstitial tissue. Abscess formation may result. With destruction of sufficient renal tissue an insufficiency may ensue with the picture of a more or less acute nephritis with hypertension, retention of nitrogen and edema. Pyelitis may complicate other types of toxemia giving a mixed picture.

Pyelitis is common enough in pregnancy. Certainly the majority of cases of this disorder do not show hypertension or other signs of toxemia. At times the blood pressure of a pregnant woman is lowered with the onset of pyelitis. Just how much of a factor renal inflammation of this kind may be in toxemia is a matter about which we have insufficient data. The question has been discussed by Peters[1]. Disease of this kind would seem to bear about the same relation to toxemia as does nephritis. An essential preliminary to an opinion here is a precise definition of terms.

Nephrosis and Pregnancy

The diagnosis of nephrosis is unusually hazardous, doubly so in pregnancy. In the general clinic most instances of so-called nephrosis if followed sufficiently long prove to be chronic glomerulonephritis. There is water retention with edema of striking degree, the continuous loss of protein by a heavy albuminuria, an inversion of the ordinary albumin-globulin ratio of the blood serum, a low serum protein, an anemia, a low blood pressure and metabolic rate, the latter not influenced by thyroid medication; a cholesterolemia, the presence of doubly refractile fat droplets and, usually, an absence of erythrocytes in the urinary sediment. In its active stages a nephrosis of this kind is incompatible with pregnancy. Patients do make a temporary or complete recovery, however. Later such may go through a pregnancy safely.

The nephrosis commonly observed in pregnancy is probably part of a general vascular disease. In theory, spasm of the renal arterioles seems the fundamental process. This leads to renal ischemia, cloudy swelling and eventually to impairment of renal function. Discussion of this variety of *nephropathia gravidarum* may best be deferred until the vascular diseases are taken up in their relation to pregnancy.

Vascular Disorders and Pregnancy – Eclampsia and Preeclampsia

For long the classification of the toxemias has been a realm of confusion, and still awaits knowledge of etiology. Witness of this is the variety of fanciful and often meaningless or misused terms applied. The subject seems to have been dealt with in a fashion too remote from pathology and the related field of internal medicine. The most significant result of recent coordinated studies in this field has been demonstration of the close relationship to if not the identity of the non-nephritic late toxemias with certain common types of vascular disease.

By far the largest proportion of these late toxemias – from 90 to 95 per cent – present a picture suggesting an acute vascular disease with hypertension, one which may subside or may become chronic. In this we may see every gradation in severity from a slight, transitory and uncomplicated rise in systolic or diastolic pressure, either or both, to some of the highest readings on record with secondary effects of the gravest sort.

Hypertension, the presenting finding, may antedate pregnancy or, as is usual, may appear during the last trimester, in many mild cases a few

days before term. In progressive cases the rise is gradual or rapid. Careful ophthalmoscopic examination may disclose arterial spasm. Other symptoms are often lacking until systolic pressures reach 170-180 mm. or more, or until diastolic pressures are at 100 mm. or more. At this point the onset of albuminuria may be rapid. This may be succeeded by diminution in urinary output, by edema of the subcutaneous tissues, and of the retina. In cases with dominant liver damage, epigastric pain and tenderness, often with icterus, are noted. Nausea and vomiting are rare. Implication of the nervous system is shown by deviations from normal mentality, exaggerated deep reflexes and by coma and convulsions. Retinal hemorrhages, patches of exudate or atrophy or edema and occasionally retinal separation are later features. Cardiac dilatation with acute pulmonary edema may occur, especially during or soon after labor. Apoplexy is more rare. Except for showing albuminuria in the advanced cases the laboratory gives little aid. There is usually no retention of urea nitrogen in the circulating blood. While the uric acid of the blood is usually elevated, creatinin is in normal concentration. The elimination of dye or of urea may be affected by the acute nephrosis which marks the more advanced instances of this disorder.

When a patient is seen in the later phases of this type of toxemia and when there is no knowledge of the sequence of events it may be difficult or impossible to differentiate it from nephritis complicated by pregnancy. We are faced with the same difficulty apart from pregnancy in advanced cases of either malady. A history of a gradual rise of blood pressure preceding the albuminuria for some time is the most important differential point. In nephritis the order of appearance of these features is reversed. The presence of anemia, of albuminuric retinitis, of nitrogen retention, of hematuria and the history of significant infections mark nephritis in contrast to vascular disease. It is possible, to be sure, to have to deal with a mixed form in which nephritis and true vascular disease coexist. Or matters may be complicated further by the occasional presence of a pyelonephritis.

Usually diagnosis is more readily made in the follow-up period. Here the picture of vascular disease or of nephritis unfolds as years go on to find final proof at necropsy.

The Effect of Vascular Disease upon Pregnancy and the Fetus

This is not as serious or abrupt as in nephritis. In the many cases in

which the pressures do not rise above 160 mm. systolic or 100 mm. diastolic, and in which albuminuria is absent or slight there is apparently little danger to the fetus and the mother may experience a second pregnancy without vascular disturbance. Such cases have been called "low reserve kidney", an unfortunate and meaningless term because the kidney has little part in the process. As Herrick and Tillman have shown[2], one-third of these patients develop hypertension in later years without demonstrable renal involvement. Even this mildest of the pregnancy hypertensions is a threat to maternal welfare.

In the more serious types of vascular toxemia, the eclampsias and preeclampsias, the results are less favorable. When convulsions occur the immediate fetal mortality is not far from 60 per cent while the immediate maternal mortality formerly averaged about 15 per cent. With recent methods of treatment this figure is undoubtedly lower. Herrick and Tillman[3] found that 22.5 per cent of the eclamptics surviving labor and the puerperium died during a follow-up period averaging 6.5 years in duration. This is a strikingly high figure. In seventy of their patients studied in subsequent pregnancies, convulsions recurred in 14.3 per cent, while a considerable number presented only a mild disturbance marked by hypertension. Corwin and Herrick[4] cited 154 multiparas with toxemia of which 13 per cent gave a history of convulsions in previous pregnancies. In the non-convulsive or preeclamptic patients, fetal mortality is about 15 per cent, while maternal deaths are few. During the follow-up period, however, more than half of these patients will show evidence of vascular disease within three years of the toxic pregnancy. At necropsy the uncomplicated cases will disclose the changes characteristic of hypertensive cardiovascular disease, rather than those of nephritis.

Many years ago Volhard[5] stated that the phenomena of the "kidney of pregnancy" could be explained best by postulating arterial spasm as a fundamental disturbance of the physiology of such patients. This theory although given general mention in the literature[6] was never developed until both obstetrician and internist began to regard the problem as not exclusively the province of the former. The group of workers at the Sloane Hospital for Women were the first to recognize and to emphasize the similarity of certain toxemias of pregnancy and vascular disease with hypertension. In 1927 Corwin and Herrick[4] published the first of a series of clinical studies bringing out this relation.

Of 165 cases of what was then called the hypertensive type of toxemia,

74 per cent exhibited some residual vascular disease in the follow-up. Of these 37 per cent had persistent hypertension. Of fifty-two cases observed in one or more pregnancies subsequent to the one first attended by this type of toxemia, fifty showed hypertension in the subsequent pregnancy. In only six of these did the blood pressure tend to become lower with succeeding pregnancies. No necropsies were reported. An elaboration of these clinical studies with like results was published in December, 1927. In 1934 Herrick and Tillman[3] reported 594 cases of toxemia followed for an average period of 5.6 years with extremes of one and twenty-two years. The mortality in this group was more than six times the average expected mortality of women of the same age. Eighty per cent of the deaths in this series were from causes within the cardiovascular-renal field. Eleven necropsies in cases long followed up were reported. As independently classified by the Department of Pathology of the College of Physicians and Surgeons, Columbia University, seven of these were examples of chronic hypertensive vascular disease; four of chronic nephritis. These findings confirmed the clinical opinion that in the late toxemias of pregnancy we have to do with two distinct groups, the larger including the eclampsias and preeclampsias having the characteristics of vascular disease with hypertension; the smaller being merely cases of nephritis or perhaps pyelonephritis complicated by pregnancy. For the first time these two groups were defined on the basis of study during the toxic pregnancy, over a long follow-up and finally by the pathologist.

In the following year McKelvey and MacMahon[7] published the results of a similar study, citing thirteen cases with necropsy after a long follow-up period. From the pathologist's report these were examples of vascular disease with hypertension apparently of the malignant variety.

In 1936 Herrick and Tillman[2] reported a study of 188 cases of the mild, non-convulsive, non-nephritic type of late toxemia, variously classified as mild toxemia, recurrent toxemia, low reserve kidney, etc. In these the antepartum blood pressure was not above 160 mm. systolic or 100 mm. diastolic and albuminuria, slight or absent. During the follow-up period about one-third had significant hypertension. Study of renal function in thirty-five follow-up cases gave no consistent variations from the normal; in other words no evidence of chronic nephritis. The clinical evidence and the single necropsy they reported tend to show that these mild toxemias vary from eclampsia and preeclampsia only in degree, not in kind. The consequences in the follow-up period and the findings at

necropsy are the same. When the entire life history of the disease is unfolded, the picture is that of a general vascular disease with hypertension.

To these studies of the internist, the ophthalmologist adds valuable support. After extensive observation, Wagener[8] concludes that the initial ophthalmic feature of the eclamptic and preeclamptic toxemias is a spasm of the retinal arteries. This may be intermittent or continuous; universal or segmental. If this spasm continues, edema, especially of the central portion of the retina, takes place, later extending over the entire fundus. Exudates, patchy degeneration, and hemorrhages follow later. If arterial spasm lasts many days, arterial changes become permanent and are accompanied by elevation of blood pressure and the entire picture of vascular disease with hypertension. In a study of 300 cases of hypertensive toxemia Hallum[9] records similar findings. Nuri[10] finds sclerotic changes in the fundus much more common in women who have a history of eclampsia. These findings have been confirmed repeatedly. Phenomena of this kind consistently and competently observed cannot be ignored. Undoubtedly what takes place in the vascular apparatus of the eye happens elsewhere in kidney, liver, brain and other organs rich in arterial blood. As in simple hyperpiesis the brunt of the disorder may be borne by different organs in different cases. In one, the renal circulation may be most profoundly involved, with albuminuria and its accompanying phenomena; in another, the liver with resulting jaundice, hepatitis or hepatic necrosis; in another the brain, giving rise to convulsions from edema or hemorrhage. On the basis of a primary vascular disturbance we may perceive a certain unity of all the late toxemias of pregnancy that are not based upon primary renal inflammation. In other words, if we yield to the temptation to make a theoretical generalization, eclampsia, preeclampsia and the milder toxemias marked by hypertension, are but variants of the same fundamental process. If this be true a most desirable simplification in our classification of these disorders is made possible as well as a new approach to their study. If we may make a common problem of vascular disease with hypertension and the late toxemias of pregnancy, we are definitely nearer finding a key which shall unlock these mysteries.

Related Factors in Hyperpiesis and Toxemias of Pregnancy

The etiology of essential hypertension is still in the region of opinion and theory. Present limited knowledge of the physiology concerned seems to place this as a disorder of homeostasis, that is, of a proper balance

of function. Concerned in this are undoubted factors associated with those endocrines having blood pressure raising hormones, the pituitary and the adrenal; perhaps also with the thyroid and gonads; also certain important neurogenic components centering about the sympathetic system. As yet one cannot fit into a consistent pattern the isolated facts bearing on the problem. One can but state a series of observations and their implications. Despite their tentative and incomplete nature one may be justified in reviewing briefly such observations in this field as seem to link the problems of hyperpiesis and the late toxemias of pregnancy.

The Pituitary in Relation to Hypertension and Toxemias of Pregnancy

The importance of the pituitary in pregnancy is probably great. The morphologic changes are profound and constant. They are sufficiently characteristic to enable a diagnosis of pregnancy to be made from the microscopic section alone[11]. The local pressure symptoms in the region of the sella are well known, the coarsening of features, the increase in size of the extremities, the abnormal bone formation all suggest unusual activity of the hypophysis. Do any characteristic variations in these findings mark toxic pregnancies?

Here Cushing's[12] finding of basophilic invasion of the nervous or posterior lobe in essential hypertension and in eclampsia must be mentioned. In Addison's disease, a condition marked by hypotension, Kraus and Traube[13], also Crooke and Russell[14] and Hawking[15] find degranulation and diminution in number of basophiles in the hypophysis—a contrast and a corroboration.

Schallock[16] noted spotty necrosis and atrophy of the anterior lobe of the hypophysis in eclampsia. Berblinger[17] finds with Cushing[12] that the hypophyses of eclamptics and hypertensives have foci of basophilic cells in the anterior lobe. Page and Sweet[18] note that hypophysectomy in dogs with hypertension produced by renal ischemia reduces arterial pressure to about normal while it appears to reduce but slightly the blood pressure of normal dogs.

Against the importance of structural changes in the hypophysis in hypertensive or eclamptic states one can collect a number of studies. It is generally recognized that there is an increased tendency toward basophilic invasion of the nervous lobe with age. Ahlström[19] found this change in 50 per cent of thirty-six cases with normal blood pressure and in 96 per

cent of twenty-five cases with hypertension. Spark[20] found such changes of little importance in eclampsia. Berblinger[17], while he considers it well established that there is a connection between the basophilic cells of the anterior lobe and high blood pressure, does not place much importance upon the invasion of the posterior lobe by basophiles. He lists ten eclamptics with absence of such invasion. Some of these showed no basophiles in the posterior lobe. Biggart[21] made serial sections of the hypophysis in eight cases of eclampsia. Not one had an unusual amount of basophilic infiltration. In ten cases of essential hypertension excessive invasion of basophiles was found in but two. He concludes this change is not more frequent in hypertension, eclampsia or marked arteriosclerosis than in controls of similar age.

More than one observer has noted a similarity between the hypertension of the menopause and that of Cushing's syndrome. Kylin[22] believes the latter but an exaggerated form of menopausal hypophyseal change. There is much that is plausible in the idea that pituitary cachexia or Simmonds' disease is the antithesis of acromegaly and of essential hypertension. In a case of this kind Kylin[22] found diminution of basophiles and increase in eosinophiles and fibrous tissue in the anterior lobe, seeing in this confirmation of Cushing's[12] idea that the basophiles are influential in states of hypertension. The fact that pituitary cachexia may follow pregnancies that are too frequent or that are attended by toxemia or by difficult or prolonged labor has given rise to the theory that an exhaustion of the hypophysis accounts for its functional failure under these conditions.

In the zone of physiology the data are even less conclusive. Schaefer[23] finds definite reduction in both systolic and diastolic pressure in the menopause following theelin therapy. The alleged favorable response of essential hypertension to small doses of x-ray applied to the pituitary and adrenal glands may have passing mention[24]. Jores[25] has expressed the opinion shared by Kylin[22] that essential hypertension is primarily a hypophyseal disease. In this the action of the pituitary is thought of as indirect through the production of an adrenalotropic hormone which produces histologic changes in the adrenals of immature mice. Jores[26] finds a substance in the blood of hypertensives causing similar changes in the adrenal medulla. Anselmino and Hoffmann [27] have described substances with like action. Jores[25] found increased amounts of this adrenalotropic hormone in Cushing's syndrome, but not in examples of renal hypertension or of

nephropathia gravidarum. Hoffmann[28] quotes Bohn, also Marx and Enfinger[29], all of whom found a blood pressure raising substance in the blood of eclamptics, presumably of hypophyseal origin. Anselmino and Hoffmann[30] find the blood and urine of hypertensives and of eclamptics contain excessive amounts of gonadotropic material. They believe that the hypertension and edema of certain toxemias result from hyperactivity of the posterior lobe of the hypophysis with its pressor and antidiuretic hormones. It is claimed that these hormones have been demonstrated in the blood of women with the so-called kidney of pregnancy. Page[31] has been unable to confirm the finding of an excess of the pressor substance of the pituitary body in eclampsia.

Kylin[32] recovered increased amounts of prolan A in the urine of patients with essential hypertension. In forty patients the amount of gonadotropic substance and the height of the blood pressure were parallel. Scarf and Israel's[33] findings do not agree. In ten of twelve hypertensives there was no increase in prolan. In two older patients, with pressures over 200 mm., prolan was increased. Studies of the hormones in toxemia of pregnancy are few. Smith and Smith[34] in studies of prolan and estrin of both urine and serum, find in normal pregnant women that the peak of prolan is in the second and third month, while the estrin is at first low but rises rapidly in the last trimester. In toxemia apart from nephritis these findings are reversed: high prolan and low estrin are found even late in pregnancy and the abnormal rise in serum prolan precedes the clinical manifestations by at least six weeks. If confirmed by further studies this observation may be significant and a method of value in disclosing a trend toward toxemia in advance of the ordinary clinical manifestations.

The constitutional type of individuals subject to hypertension and to the vascular toxemias of pregnancy is similar if not identical. The broad, heavy featured, overweight, "lateral" rather than "lineal" type is characteristic of the status apoplecticus as it is of the toxemia of pregnancy. The anthropometric study of 100 cases of toxemia by Draper[35] confirmed the clinical impression that their anatomical peculiarities were those of acromegaly and therefore suggested pituitary overactivity. Further clinical studies by Vorzimer et al[36] have had like results. While one meets both essential hypertension and toxemia in individuals of a different constitutional habitus by far the larger proportion, especially of the toxemias, occurs in those who are overweight and have the other physical stigmas described.

In the association we are considering, the adrenal gland seems bound up with the hypophysis. A definite hypertrophy of the adrenal especially of the zona fasciculata of the cortex occurs in pregnancy, to a lesser extent during menstruation and following castration. A pregnant dog survives adrenalectomy many days longer than one not pregnant. The adrenotropic influence of the pituitary seems definite. Removal or gross disease of the anterior lobe as in Simmonds' disease is followed invariably by wasting of the adrenal and by arterial hypotension. P. E. Smith[37] showed that the atrophy of the adrenal cortex which follows removal of the pituitary gland in rats can be held in abeyance by the intramuscular implantation of rats' hypophyses. Collip, Anderson and Thomson[38] claim to have isolated from the anterior lobe of the pituitary an adrenalotropic factor which when administered to 125 hypophysectomized rats caused an increase of from 50 per cent to 300 per cent in the weight of their adrenals. In hypertension Vaquez[39] noted that suprarenal hyperplasia was frequent. Aubertin and Ambard[40] noted diffuse cortical hyperplasia or adenomas of the cortex in seven of eight hypertensives. Oppenheimer and Fishberg[41] found cortical adenomas more likely to occur when high blood pressure was present. An increase in the weight of the adrenals in hypertension is noted by Philpot[42] and some observers (Wiesel, Parkinson[43]) claim to have found hyperplasia of the suprarenal medulla in hypertensives. Similar changes have been found repeatedly in subjects who during life had normal blood pressures. Evidence of increased circulation perhaps denoting increased suprarenal activity is inferred by Goldzieher and Sherman[44] from their demonstration of hypertrophy of the musculature of the adrenal veins in hypertensives. Despite the alleged demonstration of an excess of epinephrine in the blood of hypertensives by a number of observers, there is no general acceptance of the matter; it still remains the "beautiful dream of adrenalinemia" to quote T. C. Janeway[45].

More convincing is the association of hypertension with tumors of the suprarenal cortex or medulla. Here the association is direct, since successful surgical removal of the tumor generally results in a return of blood pressure to normal. The fact that the clinical picture of such suprarenal tumors may be indistinguishable from that of basophilic pituitary adenomas, a condition also marked by hypertension, may be significant. The well known effects of destructive lesions of the adrenals or of their denervation or removal in bringing about a marked hypotension is too well known to be taken up in detail. It has by no means been proved that

this is an effect of a lessened secretion of epinephrine.

Studies of the adrenals in toxemia of pregnancy are few. Fauvet[46] suggests that in toxemia the usual hypertrophy of the adrenal of pregnancy is lacking; in fact, this organ is smaller than normal. He states that hypertrophy of the suprarenals may be produced by anterior pituitary extract or by prolan. He considers toxemia of pregnancy a possible result of hypofunction of the adrenal cortex. He finds an increase of corticotropic hormone in hypertension but not in eclampsia. The theory that hyperemesis gravidarum is due to adrenal insufficiency has been advanced. Here, again, the normal hypertrophy of the cortex in pregnancy is said to be lacking. Anselmino[47] believes this form of toxemia an expression of hyperfunction on the part of some of the anterior pituitary hormones. Much of this work seems theoretical and not free from contradictions. It seems to concern the early rather than the late toxemias with which we are engaged.

The changes in the ovary in pregnancy need not be detailed: those in the thyroid are less well understood. The dependence of these glands upon the hypophysis through the tropic hormones of the latter seems definitely established and is of undoubted importance in the complicated endocrine adjustment of the pregnant woman to the demands of the fetus. One other organ of internal secretion – the placenta – must have consideration. It produces prolan, estrone, progesterone and possibly other hormones (Kurzrok[48]). The effect of this new organ upon the maternal economy must be profound. In perversion of its functions at some future time one may find the origin of some of the disturbances of pregnancy.

In this cursory review of recent endocrinological study in the related fields of essential hypertension and toxemia one is impressed with the truth of the dictum that any biologic problem sufficiently pursued leads to infinity. Repeatedly we are faced with the question which is cause and which effect? The complexities and occasional contradictions unearthed are baffling and forbid present acceptance of the theory of the endocrine origin of the disorders discussed. And yet through all this maze runs such a strong thread of plausibility that one cannot abandon it. Some day it may lead to truth. In what Kurzrok[48] terms the equilibrated organism and Cannon[49] the individual in homeostasis lies health. It is possible that in a lack of balanced function of the pituitary-adrenal complex and of its sympathetic nervous connections, influenced by the ovary, the thyroid

and in pregnancy by the placenta, we may uncover the nature of at least some of the various disturbances we now call the toxemias of pregnancy. As yet, however, we cannot subscribe without reservations to that which often seems to have more of shadow than of substance.

While awaiting the essential additions to our knowledge of physiology the clinician must continue his bedside observations which should include the entire life history of the patient and of her disease. Already in collaboration with the obstetrician and the pathologist much light has been thrown upon the problem and more is promised.

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